CAIO RODRIGUES MONTEIRO

EFEITOS DA SAZONALIDADE E DA SUPLEMENTAÇÃO COM METIONINA NA PRODUÇÃO, COMPOSIÇÃO DO LEITE E METABOLISMO DE VACAS LEITEIRAS

LAVRAS – MG
2025

CAIO RODRIGUES MONTEIRO

EFEITOS DA SAZONALIDADE E DA SUPLEMENTAÇÃO COM METIONINA NA PRODUÇÃO, COMPOSIÇÃO DO LEITE E METABOLISMO DE VACAS LEITEIRAS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do programa de Pós-Graduação em Zootecnia, área de concentração em Nutrição e Produção de Ruminantes, para a obtenção do título de Doutor.

Orientadora
Prof. DSc. Marina de Arruda Camargo Danés

LAVRAS – MG
2025

Ficha Catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Monteiro , Caio Rodrigues.
Efeitos da sazonalidade e da suplementação com metionina na produção, composição do leite e metabolismo de vacas leiteiras / Caio Rodrigues Monteiro . - 2025.
158 p.

Orientadora: Marina de Arruda Camargo Danés

Tese (Doutorado) - Universidade Federal de Lavras, 2025.
Bibliografia.

1. Composição do leite. 2. Cosinor. 3. Estresse térmico . 4. Metionina protegida. 5. Ritmos circanuais. I. Danés, Marina de Arruda Camargo . II. Universidade Federal de Lavras. III. Título.

CAIO RODRIGUES MONTEIRO

EFEITOS DA SAZONALIDADE E DA SUPLEMENTAÇÃO COM METIONINA NA PRODUÇÃO, COMPOSIÇÃO DO LEITE E METABOLISMO DE VACAS LEITEIRAS

EFFECTS OF SEASONALITY AND METHIONINE SUPPLEMENTATION ON MILK YIELD, MILK COMPOSITION, AND METABOLISM IN DAIRY COWS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do programa de Pós-Graduação em Zootecnia, área de concentração em Nutrição e Produção de Ruminantes, para a obtenção do título de Doutor.

APROVADA em 28 de novembro de 2025.

DSc. Marina de Arruda Camargo Danés	UFLA
DSc. Aleksandro Schafer da Silva	UDESC
DSc. Daniel de Noronha Figueiredo Vieira da Cunha	UFSJ
DSc. Juliana Petrini	CLÍNICA DO LEITE
DSc. Rafael Fernandes Leite	UFSJ

Documento assinado digitalmente
gov.br
MARINA DE ARRUDA CAMARGO DANES
Data: 25/02/2026 11:00:01-0300
Verifique em https://validar.iti.gov.br

Orientadora
Prof.ª DSc. Marina de Arruda Camargo Danés

LAVRAS – MG
2025

Dedico este trabalho à Nossa Senhora Aparecida, por sua intercessão, proteção e por ter iluminado meu caminho durante toda esta jornada

AGRADECIMENTOS

Primeiramente, a Deus, que não apenas hoje, mas ao longo de todos os momentos da minha vida, esteve presente, permitindo-me alçar voos cada vez mais altos.

À minha família, em especial à Juliana, que sempre esteve ao meu lado, festejando os bons momentos, confortando-me e ajudando-me a enfrentar os ruins. Pela paciência e compreensão diante dos momentos de ausência que se fizeram necessários para alcançar mais este objetivo pessoal. À nossa pequena Giovana, que surgiu durante a fase final do doutorado e se tornou uma grande fonte de motivação.

À Universidade Federal de Lavras (UFLA) e ao Programa de Pós-Graduação em Zootecnia (PPGZ/UFLA), pela oportunidade de realizar este doutorado e por toda a estrutura, acolhimento e excelência na formação científica, desde o mestrado até o presente momento. Ao Departamento de Zootecnia (DZO/UFLA), pela infraestrutura, apoio técnico e ambiente colaborativo que tornaram possível o desenvolvimento deste trabalho.

À Universidade Federal de São João del-Rei (UFSJ) e ao Departamento de Zootecnia (DEZOO/UFSJ), pelo incentivo constante à qualificação de seu corpo técnico e pelas condições concedidas para a realização deste doutorado.

À minha orientadora, Prof.ª Marina de Arruda Camargo Danés, que esteve presente durante todo esse processo de formação acadêmica, contribuindo com valiosos conhecimentos e experiências para que este trabalho se concretizasse. Agradeço, especialmente, pela inspiração e incentivo, inclusive nos momentos mais desafiadores, mesmo quando eu próprio já não acreditava.

À Fazenda Fazendinha (Três Corações – MG), pelo fornecimento dos animais, pela infraestrutura disponibilizada para a realização do experimento e pela receptividade aos alunos do nosso grupo de pesquisa. A parceria com essa fazenda foi essencial para o desenvolvimento do estudo. Acredito firmemente que a pesquisa deve ser conduzida para resolver problemas reais, e a participação da iniciativa privada nesse processo é fundamental.

À Evonik Operations GmbH (Alemanha), pela concessão do produto Mepron® e pelo apoio financeiro que viabilizou o experimento com suplementação de metionina protegida. À PECPRO Nutrição Animal (Alfenas – MG), por ter intermediado os contatos necessários e operacionalizado a produção do suplemento utilizado no experimento.

À Bioclin Química Clínica (Brasil), pelo fornecimento dos reagentes utilizados nas análises bioquímicas, fundamentais para o desenvolvimento deste estudo. À Clínica do Leite (Piracicaba – SP) pela realização das análises de leite do experimento de campo.

À Associação Brasileira dos Criadores de Girolando, pelo fornecimento dos dados utilizados na pesquisa sobre a sazonalidade da composição do leite no Brasil. Ao Dr. Isaac J. Salfer (University of Minnesota), pela generosidade e colaboração científica na realização deste trabalho.

Aos amigos e colegas de jornada, que com amizade, paciência e incentivo tornaram os dias mais leves. Em especial ao nosso grupo de pesquisa INPPAR, é uma honra muito grande poder dizer que fiz parte do mesmo grupo de pessoas que tanto admiro.

A todos que, de alguma forma, contribuíram para a concretização deste trabalho, deixo aqui meu mais sincero agradecimento.

RESUMO

A produção e a composição do leite em sistemas tropicais variam ao longo do ano e são afetadas por períodos críticos de estresse térmico. Esta tese reuniu dois estudos complementares. No primeiro, objetivou-se caracterizar ritmos circanuais de gordura, proteína e contagem de células somáticas (CCS) no Brasil e comparar a contribuição de sazonalidade e variáveis econômicas, utilizando dados mensais de tanques (2013–2023) de seis estados (792 observações estado-mês) e registros Girolando (10.987 observações; 718 vacas; 41 rebanhos), com modelos lineares mistos cosinor de 12 meses (mercado, rebanho e vaca) e modelos com preços de leite, milho e soja. Detectaram-se ritmos anuais significativos ($P < 0,001$) para gordura, proteína e CCS em todos os estados, e a sazonalidade explicou a maior parcela da variação, com ganho mínimo ao adicionar variáveis econômicas ($\Delta R^2 \leq 0,01$). Em rebanhos, 65,9% foram rítmicos para proteína, 31,7% para gordura, 34,1% para produção e 22,0% para CCS; em vacas, observaram-se ritmos em 46,2% (proteína), 20,5% (gordura), 51,6% (produção) e 22,2% (CCS), confirmando padrões preservados entre níveis organizacionais. No segundo estudo, avaliou-se a suplementação com metionina protegida da degradação ruminal (MPR) em vacas Holandesas primíparas em meio de lactação durante o verão, sob estresse térmico (THI > 68 em 68,3% das horas; THI médio = 70,6), em ensaio de 9 semanas com 80 vacas (182 ± 64 dias em lactação; 42,9 ± 4,7 kg/d) recebendo dieta controle ou MPR (0,75 g/kg de MS; ~20 g/vaca/d) e coletas nas semanas 3, 6 e 9. A MPR aumentou a produção de leite em 2,0 kg/d (44,9 vs. 42,9), o rendimento de proteína em 50 g/d (1.464 vs. 1.414), de lactose em 108 g/d (2.109 vs. 2.001) e de sólidos totais em 176 g/d (5.331 vs. 5.155), além de elevar insulina (0,52 vs. 0,35 ng/mL), reduzir glicose (39,3 vs. 43,2 mg/dL), aumentar FRAP (32,9 vs. 28,5 μM) e reduzir malondialdeído (2,48 vs. 2,78 nmol/mL), sem aumento consistente da temperatura vaginal média. Conclui-se que os ritmos circanuais de composição do leite e CCS existem e são diferentes entre estados e que a MPR melhora produção e sólidos e favorece o estado endócrino e redox de vacas primíparas sob calor, sendo estratégia promissora para mitigar perdas no verão em sistemas leiteiros tropicais.

Palavras-chave: composição do leite; cosinor; estresse térmico; metionina protegida; ritmos circanuais

ABSTRACT

Milk production and composition in tropical systems vary throughout the year and are affected by critical periods of heat stress. This thesis comprised two complementary studies. In the first, we characterized circannual rhythms of milk fat, protein, and somatic cell count (SCC) in Brazil and compared the relative contribution of seasonality and economic variables using monthly bulk-tank data (2013–2023) from six states (792 state-month observations) and Girolando records (10,987 observations; 718 cows; 41 herds), fitting 12-mo cosinor linear mixed models at market, herd, and cow levels, as well as models including milk, corn, and soybean prices. Significant annual rhythms ($P < 0.001$) were detected for fat, protein, and SCC in all states, and seasonality explained most of the variation, with minimal gains from adding economic variables ($\Delta R^2 \leq 0.01$). At the herd level, 65.9% were rhythmic for protein, 31.7% for fat, 34.1% for milk yield, and 22.0% for SCC; at the cow level, rhythms were observed in 46.2% (protein), 20.5% (fat), 51.6% (milk yield), and 22.2% (SCC), confirming patterns preserved across organizational levels. In the second study, we evaluated rumen-protected methionine (RPM) supplementation in mid-lactation primiparous Holstein cows during summer heat stress (THI > 68 for 68.3% of monitored hours; mean THI = 70.6) in a 9-wk trial with 80 cows (182 ± 64 DIM; 42.9 ± 4.7 kg/d) fed a control diet or RPM (0.75 g/kg diet DM; ~20 g/cow/d), with sampling in weeks 3, 6, and 9. RPM increased milk yield by 2.0 kg/d (44.9 vs. 42.9), protein yield by 50 g/d (1,464 vs. 1,414), lactose yield by 108 g/d (2,109 vs. 2,001), and total solids yield by 176 g/d (5,331 vs. 5,155), increased insulin (0.52 vs. 0.35 ng/mL), reduced plasma glucose (39.3 vs. 43.2 mg/dL), increased FRAP (32.9 vs. 28.5 μM), and reduced malondialdehyde (2.48 vs. 2.78 nmol/mL), without a consistent increase in mean vaginal temperature. In conclusion, circannual rhythms in milk composition and SCC are present and differ among Brazilian states, and RPM improves milk and solids yields while favoring endocrine and redox status in primiparous cows under heat stress, representing a promising strategy to mitigate summer losses in tropical dairy systems.

Keywords: circannual rhythms; cosinor; heat stress; milk composition; rumen-protected methionine

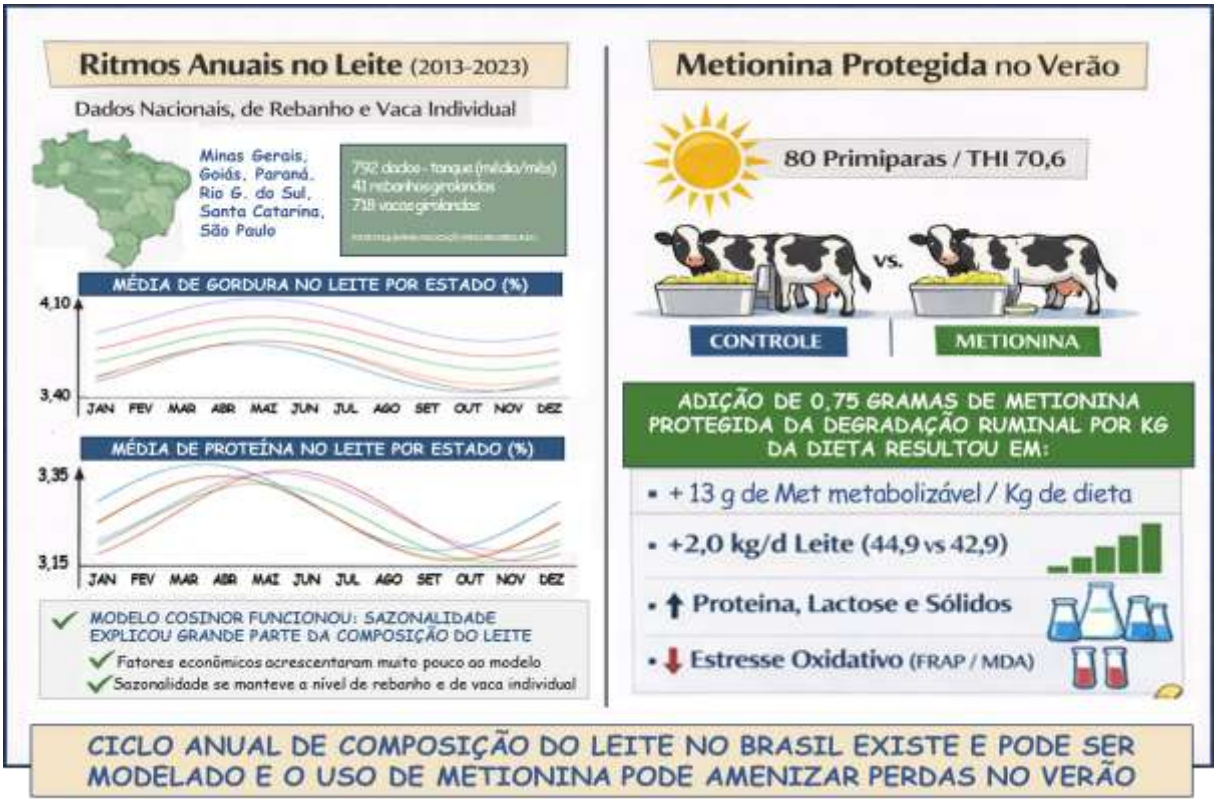
Efeitos da sazonalidade e da suplementação com metionina na produção, composição do leite e metabolismo de vacas leiteiras

Elaborado por **Caio Rodrigues Monteiro** e orientado por **Marina de Arruda Camargo Danes**

A produção e a qualidade do leite no Brasil mudam ao longo do ano e tendem a cair nos períodos mais quentes. Isso afeta a renda do produtor, o planejamento das indústrias e a qualidade do alimento que chega ao consumidor. Esta tese reuniu dois estudos para compreender essas variações e indicar uma forma de reduzir perdas no verão, período especialmente desafiador devido ao calor.

No primeiro estudo, analisaram-se dados mensais de qualidade do leite de seis estados brasileiros entre 2013 e 2023, além de registros de rebanhos e vacas da raça Girolando. Utilizou-se um método estatístico que caracteriza ciclos anuais para avaliar gordura, proteína e contagem de células somáticas. Foram identificados ciclos anuais claros, consistentes e diferentes entre os estados, e a sazonalidade explicou a maior parte das mudanças observadas, enquanto fatores econômicos (como preços do leite e de insumos) acrescentaram pouca explicação adicional. Assim, conclui-se que a sazonalidade é um componente real e importante da variação da qualidade do leite e deve ser considerada na interpretação de resultados e no planejamento de ações tanto na fazenda quanto na indústria.

No segundo estudo, avaliou-se uma estratégia nutricional durante o verão em vacas Holandesas primíparas, categoria mais sensível ao estresse por calor. O ambiente apresentou calor suficiente para causar estresse térmico na maior parte do tempo. As vacas que receberam metionina protegida produziram, em média, 2,0 kg a mais de leite por dia (44,9 vs. 42,9 kg/dia) e aumentaram a produção de proteína, lactose e sólidos totais. Além disso, apresentaram melhora em marcadores sanguíneos de proteção contra estresse oxidativo. Em conjunto, os resultados mostram que a sazonalidade pode orientar decisões de manejo e políticas de pagamento por qualidade e que a metionina protegida é uma alternativa promissora para melhorar o desempenho produtivo no verão.



Tese de Doutorado em Zootecnia na UFLA, defendida em 28/11/2025.

INDICADORES DE IMPACTO

Este trabalho, composto por dois estudos complementares, apresenta impactos sociais, tecnológicos, econômicos e ambientais, diretos e potenciais, para a cadeia produtiva do leite em sistemas tropicais no Brasil. O primeiro estudo, *"Annual rhythms of milk composition in dairy cattle in Brazil"*, analisou bases nacionais de qualidade do leite vinculadas ao Programa Nacional de Qualidade do Leite (PNQL) do Ministério da Agricultura e Pecuária, bem como dados setoriais da Associação Brasileira dos Criadores de Girolando, gerando evidências aplicáveis à previsão da variação sazonal da composição do leite entre regiões. Esses resultados apresentam impacto tecnológico e econômico potencial ao subsidiar programas de pagamento por qualidade, planejamento de captação e produção, gestão de processos industriais e ações de controle de qualidade por cooperativas, indústrias e órgãos reguladores, contribuindo para maior eficiência e previsibilidade na cadeia. O segundo estudo, *"Response of mid-lactation primiparous Holstein cows to the supplementation of rumen-protected methionine during the summer"*, foi conduzido em fazenda comercial (Fazenda Fazendinha, Três Corações, MG), em parceria entre a universidade e o setor produtivo, e avaliou a suplementação com Metionina visando a resiliência metabólica de vacas Holandesas primíparas durante o verão. Os resultados têm impacto social e tecnológico potencial ao apoiar recomendações de manejo nutricional para redução de perdas produtivas e de desequilíbrios fisiológicos sob estresse térmico, com implicações para a sustentabilidade dos sistemas e para a adaptação frente a cenários de aquecimento global. O caráter extensionista do trabalho evidencia-se pela interação entre universidade, produtores e instituições setoriais e governamentais, com participação de público externo à UFLA e geração de conhecimento aplicável a demandas reais do setor, além de impactos indiretos para consumidores por meio do fortalecimento da qualidade, da eficiência e da regularidade da oferta de matéria-prima ao longo do ano. À luz da Política Nacional de Extensão Universitária, os impactos se enquadram principalmente nas áreas temáticas Saúde, Meio ambiente e Tecnologia e produção, com interfaces em Trabalho, considerando os efeitos sobre eficiência e sustentabilidade da atividade leiteira. Por fim, os resultados dialogam com os Objetivos de Desenvolvimento Sustentável da Organização das Nações Unidas, especialmente ODS 2 (fome zero e agricultura sustentável), ODS 12 (consumo e produção responsáveis), ODS 13 (ação contra a mudança global do clima) e ODS 17 (parcerias), ao integrar dados em larga escala e evidências experimentais aplicáveis para orientar manejo, nutrição e políticas e estratégias de qualidade em sistemas leiteiros tropicais.

IMPACT INDICATORS

This work, comprising two complementary studies, presents direct and potential social, technological, economic, and environmental impacts on the dairy production chain in tropical systems in Brazil. The first study, “Annual rhythms of milk composition in dairy cattle in Brazil”, analyzed national milk-quality databases linked to the National Milk Quality Program (PNQL) of the Brazilian Ministry of Agriculture and Livestock, as well as sectoral data from the Brazilian Girolando Breeders Association, generating evidence applicable to forecasting seasonal variation in milk composition across regions. These findings have potential technological and economic impact by supporting quality-based payment programs, milk collection and production planning, industrial process management, and quality-control actions by cooperatives, dairy industries, and regulatory agencies, thereby contributing to greater efficiency and predictability along the supply chain. The second study, “Response of mid-lactation primiparous Holstein cows to the supplementation of rumen-protected methionine during the summer”, was conducted on a commercial dairy farm (Fazenda Fazendinha, Três Corações, Minas Gerais, Brazil) in partnership between the university and the productive sector, and evaluated methionine supplementation aimed at improving the metabolic resilience of primiparous Holstein cows during the summer. The results have potential social and technological impact by supporting nutritional management recommendations to reduce production losses and physiological imbalances under heat stress, with implications for system sustainability and adaptation under global warming scenarios. The extension/outreach nature of the work is evidenced by the interaction among the university, producers, and sectoral and governmental institutions, with participation of stakeholders external to UFLA and the generation of knowledge applicable to real demands of the dairy sector, as well as indirect benefits to consumers through strengthened product quality, improved efficiency, and more regular year-round supply of raw milk. In line with the Brazilian National Policy for University Extension, the impacts fall primarily within the thematic areas of Health, Environment, and Technology and Production, with interfaces with Work when considering effects on efficiency and sustainability of dairy activity. Finally, the results align with the United Nations Sustainable Development Goals, especially SDG 2 (Zero Hunger and sustainable agriculture), SDG 12 (Responsible consumption and production), SDG 13 (Climate action), and SDG 17 (Partnerships), by integrating large-scale data and applicable experimental evidence to inform management, nutrition, and quality-related policies and strategies in tropical dairy systems.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

A produção de leite em regiões tropicais é influenciada por fatores ambientais, fisiológicos e nutricionais que afetam a produção e a composição do leite, tornando essencial compreender esses mecanismos para sustentar a competitividade e a sustentabilidade da pecuária leiteira tropical.

A composição do leite varia ao longo do ano, refletindo a interação entre sazonalidade ambiental, disponibilidade e qualidade de alimentos e ajustes fisiológicos (NIU et al., 2014; BERNABUCCI et al., 2015). Evidências indicam que a lactação bovina também é modulada por ritmos biológicos endógenos (LINCOLN et al., 2006), sincronizados principalmente pelo fotoperíodo, via melatonina, com influência sobre hormônios associados à lactogênese e ao metabolismo mamário (CHEW et al., 1979; DAHL et al., 2000; PICCIONE et al., 2012).

A caracterização desses ritmos pode ser realizada por meio da ritmometria cosinor, um modelo cronobiológico utilizado para quantificar padrões temporais (BOURDON et al., 1995; KOUKKARI; SOTHERN, 2006). Estudos em regiões de clima temperado identificaram ritmos anuais consistentes na produção e na composição do leite (SALFER et al., 2019, 2020), porém tais padrões ainda são pouco avaliados em regiões tropicais e subtropicais.

No Brasil, o Programa Nacional de Qualidade do Leite (PNQL) disponibiliza bancos de dados extensos, ainda pouco explorados para quantificar e comparar ritmos anuais da composição do leite entre regiões. Assim, a descrição desses padrões em rebanhos brasileiros pode fornecer um referencial importante para interpretar variações sazonais e orientar decisões na fazenda e na indústria.

Dentro dessa dinâmica anual, os meses mais quentes elevam o risco de perdas produtivas e alterações fisiológicas. Entre os fatores que mais comprometem a produção, a saúde e a reprodução de vacas leiteiras está o estresse térmico (BECKER et al., 2020; BAGATH et al., 2019). O aumento das temperaturas ambientais reduz a ingestão de matéria seca e altera a partição de nutrientes, afetando a homeostase metabólica e comprometendo a eficiência produtiva (BAUMGARD; RHOADS, 2013). Reduzir a sazonalidade da produção de leite é um desafio para a indústria de laticínios, pois menor sazonalidade permite uma oferta menos volátil de matéria-prima.

Embora medidas físicas de resfriamento sejam amplamente adotadas para mitigar o calor (LEGRAND et al., 2011), estratégias nutricionais podem complementar a mitigação do estresse térmico. Entre elas, a suplementação com metionina protegida da degradação ruminal pode favorecer a síntese proteica e a manutenção do metabolismo oxidativo de vacas sob desafio térmico (MCFADDEN et al., 2020; BATISTEL et al., 2017; OSORIO et al., 2013); contudo, são necessárias mais investigações para confirmar essa eficiência, especialmente em vacas primíparas sob estresse térmico prolongado (CASTRO-MONTOYA; COREA, 2020).

Esta tese reúne dois estudos complementares: (i) caracterização de ritmos anuais da composição do leite no Brasil por ritmometria; e (ii) avaliação da suplementação com metionina protegida no verão em vacas Holandesas primíparas sob estresse térmico. Em conjunto, os trabalhos têm por objetivo ampliar a compreensão da variabilidade temporal da composição do leite e de estratégias potenciais para mitigar perdas em períodos críticos do ano.

2 REFERENCIAL TEÓRICO

2.1 Produção e composição do leite no Brasil

O Brasil foi o terceiro maior produtor mundial de leite em 2024, com uma produção de aproximadamente 25,28 bilhões de litros de leite inspecionado e uma estimativa de 35,7 bilhões de litros de produção total, incluindo o volume não formalizado (IBGE, 2025; EMBRAPA, 2025). A cadeia leiteira ocupa papel central na economia agropecuária nacional, distribuída em mais de um milhão de propriedades e gerando impacto socioeconômico, principalmente nas regiões Sul, Sudeste e Centro-Oeste (BRASIL, 2024).

Apesar dessa expressividade, o crescimento da produção tem sido modesto. Entre 2013 e 2020, a expansão média anual foi de apenas 0,5%, muito abaixo da taxa de 4,3% observada entre 2000 e 2013, período impulsionado pela elevação do consumo interno de lácteos (CARVALHO, 2021). Os dados mais recentes reforçam esse cenário: em 2024, a produção cresceu apenas 1,4% frente a 2023 (IBGE, 2024).

O Censo Agropecuário de 2017 já evidenciava limitações estruturais, como 1,176 milhão de produtores, produtividade média de 2.069 litros/vaca/ano e 28% de produção informal (IBGE, 2017). Mesmo assim, o setor apresenta sinais de modernização, incluindo aumento da escala produtiva e maior adoção de tecnologias. De fato, o volume de leite proveniente de sistemas confinados passou de 16% para 35% entre 2019 e 2021, apesar de pequena variação no número de produtores adotando esse sistema, indicando concentração produtiva crescente (CARVALHO, 2021).

Estudos recentes reforçam essa transição em direção a sistemas mais tecnificados. Santos et al. (2024) registraram, em levantamento com 669 amostras de leite de tanque no Rio Grande do Sul, que gordura, proteína e lactose apresentaram valores médios dentro dos padrões normativos, mas 31,24% das amostras estavam abaixo do mínimo exigido para lactose pela IN 76, refletindo fragilidades ainda presentes em sistemas extensivos. Garcia et al. (2024) também mostraram que caseína, proteína e lactose apresentam correlação positiva com a estabilidade do leite, reforçando a importância do manejo nutricional e sanitário na qualidade industrial.

Por outro lado, evidências de longo prazo apontam evolução gradual da composição em rebanhos tecnificados. Em análise de 23 anos de controle leiteiro no Paraná, Portes et al. (2024) observaram aumentos consistentes nos teores médios de

gordura e proteína, especialmente após a ampliação dos programas de pagamento por qualidade.

Esses achados convergem com a tendência nacional de melhoria da qualidade físico-química e microbiológica do leite, impulsionada por maior adoção de boas práticas de manejo, avanços em resfriamento e maior controle sanitário. As Instruções Normativas nº 76 e 77 (BRASIL, 2018) foram determinantes nesse processo ao elevar os padrões mínimos de CCS, CBT, temperatura e composição. Em consequência, atributos como gordura, proteína e caseína ganharam importância crescente na remuneração do produtor, consolidando sua centralidade na eficiência industrial e na competitividade do setor (CARVALHO et al., 2021).

2.2 Fatores que afetam a composição do leite

A composição do leite constitui um dos indicadores mais sensíveis da interação entre genética, nutrição, ambiente, fisiologia e sanidade. Além de determinar o valor nutricional, a composição pode influenciar diretamente o rendimento industrial e a remuneração do produtor.

A composição do leite varia ao longo da lactação, entre indivíduos, entre rebanhos e de acordo com fatores fisiológicos, nutricionais, ambientais e sanitários. A gordura e a proteína são os componentes de maior importância econômica, pois definem o rendimento industrial de queijos, manteiga, iogurtes e outros derivados. A gordura tem maior amplitude de variação e está fortemente associada às condições nutricionais e ruminais, enquanto a proteína, por ser componente estrutural, é mais estável, porém sensível ao balanço nitrogenado e ao metabolismo energético.

A composição do leite é influenciada por uma série de fatores não nutricionais. Entre esses determinantes se destacam características raciais e produtivas, parâmetros genéticos, estágio de lactação, ritmos biológicos associados à estação do ano, estresse térmico e a saúde da glândula mamária. Embora esses fatores atuem de forma independente, suas interações explicam grande parte da variabilidade observada nos teores de gordura, proteína e lactose em rebanhos comerciais, tornando indispensável compreendê-los para interpretar adequadamente resultados analisados em fazendas e estabelecer metas realistas de sólidos.

Diferenças raciais na composição do leite são conhecidas há décadas (LABEN, 1963), e refletem não apenas a fisiologia própria de cada raça, mas, sobretudo, o histórico

de seleção. Vacas Jersey, por exemplo, produzem leite com maior teor de sólidos do que vacas Holandesas (MEIRELLES et al., 2016), resultado esperado de uma trajetória genética onde a raça Holandesa foi intensamente selecionada para produção total de sólidos por lactação, e não para porcentagem por litro (GIBSON, 1989). Entretanto, isolar o efeito da raça é difícil, pois o nível de produção apresenta correlação genética negativa com os teores de gordura e proteína (WILCOX, 1992). Estudos com diferentes composições entre Holandês e Gir (CHALFUN et al., 2009) mostram que diferenças aparentes no teor de sólidos desaparecem quando a produção de leite é usada como covariável, indicando que parte das diferenças atribuídas à raça pode na verdade refletir efeitos do nível produtivo. Em contrapartida, comparações envolvendo animais de baixa produção, como relatado por Verneque et al. (2006), indicam que raças como o Gir podem apresentar, de fato, maior gordura quando o nível produtivo é semelhante entre grupos. Essa combinação de evidências confirma que raça e produção interagem de forma estreita, e que avaliações isoladas podem levar a conclusões equivocadas sobre o verdadeiro potencial de sólidos de cada população.

Além das diferenças raciais, o componente genético dentro da própria raça também exerce efeito sobre a composição do leite. Características como teor de gordura e proteína apresentam herdabilidade moderada a alta (WILCOX, 1992), o que permite progresso genético consistente quando esses componentes são incluídos nos objetivos de seleção. A trajetória da raça Holandesa nos Estados Unidos exemplifica essa relação: apesar de o teor de gordura e proteína ter permanecido praticamente estável por décadas, a produção absoluta desses componentes cresceu de maneira expressiva entre 1960 e 2019, acompanhando o aumento da produção de leite por lactação (CDCB, 2021). Apenas a partir de 2014 observou-se aumento significativo nos teores percentuais, uma mudança que coincide com estratégias de seleção mais equilibradas entre volume e sólidos. Para países como o Brasil, onde o valor pago pelo leite ainda privilegia o volume, mas onde bônus por qualidade já são realidade para muitos produtores (CARVALHO et al., 2021), incorporar critérios relacionados à composição na seleção se torna cada vez mais estratégico, sobretudo considerando que os efeitos genéticos se manifestam de forma cumulativa e de longo prazo.

O estágio da lactação também provoca mudanças previsíveis na composição do leite. Os teores de gordura e proteína tendem a ser mais elevados nos primeiros dias, reduzem no período em torno do pico de produção devido ao efeito de diluição e voltam a aumentar à medida que a produção cai no final da lactação (SANTOS; FONSECA,

2019). A lactose, embora apresente menor variabilidade, costuma acompanhar o padrão de produção de leite, caindo progressivamente conforme o tecido secretor reduz sua atividade (COSTA et al., 2019). Variáveis relacionadas ao metabolismo do nitrogênio, como o NUL, refletem o consumo de proteína e a relação proteína:energia, e frequentemente atingem seus valores mais altos semanas após o pico (DOSKA, 2012). Essa dinâmica fisiológica reforça que comparações entre grupos de animais devem sempre considerar o estágio da lactação para evitar interpretações equivocadas sobre o potencial individual ou do lote.

Além dos determinantes biológicos ligados à fisiologia da lactação, estudos recentes revelam que a composição do leite apresenta um ritmo biológico circanual, provavelmente regulado pelo fotoperíodo. Evidências provenientes dos Estados Unidos demonstram que gordura e proteína seguem um padrão anual consistente, alinhando-se aos solstícios, com maiores concentrações nos meses de dias mais curtos (SALFER et al., 2019; 2020). Esses padrões foram quantificados por modelos harmônicos (BOURDON et al., 1995) e persistem mesmo quando controlados fatores como temperatura e manejo, sugerindo a existência de um regulador endógeno sincronizado por sinais luminosos, similar ao observado em espécies sazonais como ovinos e equinos (LINCOLN et al., 2003; WEBSTER et al., 1991; HAZLERRIG et al., 2018). Do ponto de vista prático, isso significa que parte da variação anual observada nos sólidos pode não ser atribuída diretamente ao manejo ou à nutrição e que intervenções realizadas em determinados meses podem parecer mais ou menos eficientes devido à fase do ritmo biológico em que o animal se encontra.

Outro fator de grande impacto é o estresse térmico, considerado um dos principais limitadores de desempenho em regiões de clima quente (ST-PIERRE et al., 2003). A seleção para alta produção elevou o calor metabólico gerado pelos animais, aumentando a susceptibilidade ao calor (SPIERS et al., 2004). As quedas de produção observadas em condições de estresse térmico podem chegar a 40% (WEST, 2003), e se originam tanto da redução do consumo, quanto de alterações metabólicas profundas que afetam síntese de lactose, uso de glicose, metabolismo proteico e atividade hormonal (MOHAMMED; JOHNSON, 1985; ALAMER, 2011; RHOADS et al., 2009; WHEELOCK et al., 2010). Embora os efeitos sobre o teor de gordura sejam variáveis entre estudos (RHOADS et al., 2009; WHEELOCK et al., 2010; COWLEY et al., 2015), a redução no teor de proteína é mais consistente, e reflete, em grande parte, limitação de aminoácidos para síntese na glândula mamária (COWLEY et al., 2015). Estudos recentes mostram que o resfriamento

melhora o status antioxidante, aumenta a disponibilidade de aminoácidos essenciais e eleva o teor de proteína do leite (GAO et al., 2021). Além disso, o estresse térmico altera o metabolismo do nitrogênio, elevando o NUL (GAO et al., 2017), o que sugere menor eficiência de utilização de aminoácidos e maior mobilização sistêmica.

A saúde da glândula mamária completa esse conjunto de fatores não nutricionais. A mastite, especialmente na forma subclínica e de alta prevalência (BUSANELLO et al., 2017), altera profundamente a composição do leite. A inflamação compromete a integridade epitelial e modifica a permeabilidade dos tecidos, reduzindo a síntese de lactose, deslocando esse açúcar para sangue e urina e proporcionando substrato para patógenos (COSTA et al., 2019). Como a lactose determina o volume de leite por sua função osmótica, sua redução leva também a queda significativa na produção. Alterações em proteína e gordura dependem tanto da intensidade do processo inflamatório quanto da redução proporcional da produção; aumentos no teor de gordura, por exemplo, podem refletir efeito de concentração (MACHADO et al., 2000; PAIXÃO et al., 2017). A alta CCS, portanto, não apenas reduz sólidos verdadeiros, mas compromete a funcionalidade industrial do leite.

Embora os fatores não nutricionais estabeleçam os limites fisiológicos, os determinantes nutricionais modulam diretamente a síntese de gordura e proteína do leite. A gordura é o componente mais responsivo ao manejo nutricional, principalmente devido à sensibilidade das vias ruminais de fermentação. A ingestão de amido degradável e a disponibilidade de fibra fisicamente efetiva (FDNfe) modulam o pH ruminal e o perfil de ácidos graxos voláteis, influenciando a produção de acetato e butirato, precursores da síntese de novo de ácidos graxos (BRADFORD; ALLEN, 2004; ZEBELI et al., 2012; WOOLPERT et al., 2017). Quando o ambiente ruminal favorece a rota alternativa de biohidrogenação, especialmente em condições de baixo pH ou excesso de ácidos graxos insaturados, ocorre formação de intermediários como o CLA trans-10, cis-12, potente inibidor da síntese de gordura na glândula mamária (BAUMAN; GRIINARI, 2001; LOCK et al., 2006; JENKINS, 2014). Essa depressão da gordura (DGL) representa uma das respostas nutricionais mais estudadas, sendo classicamente associada a dietas ricas em amido, baixa FDNfe ou ingestão elevada de lipídios insaturados.

Variáveis de manejo, como frequência de alimentação, qualidade da mistura da TMR e espaço de cocho, também influenciam a estabilidade ruminal e, consequentemente, o teor de gordura (WOOLPERT et al., 2017; OETZEL, 2007). A seleção contra partículas longas reduz o teor de FDNfe ingerido, aumentando o risco de

acidose e de DGL, enquanto uma dieta bem misturada reduz variações individuais (MILLER-CUSHON; DEVRIES, 2017; MAULFAIR et al., 2013).

A proteína do leite, por sua vez, depende principalmente da produção de proteína microbiana (Pmic) no rúmen e do aporte de aminoácidos essenciais provenientes da proteína metabolizável (PM). A síntese de Pmic, responsável por 45–55% da PM absorvida (NASEM, 2021), é influenciada pela energia fermentável, pela degradabilidade do amido, pela digestibilidade da FDN e pela disponibilidade de proteína degradável no rúmen (OBA; ALLEN, 2003; DEPETERS; CANT, 1992; SCHWAB; BRODERICK, 2017). Desbalanço entre carboidratos e proteína degradável pode reduzir a síntese microbiana e, em casos de acidose subclínica, comprometer ainda mais a utilização de nitrogênio (ALLEN, 1999). Aminoácidos como lisina, metionina e histidina são frequentemente limitantes para síntese de proteína do leite, justificando o uso de suplementos ruminalmente protegidos (SCHWAB; BRODERICK, 2017; RIUS et al., 2010).

Níveis de NUL refletem essa interação entre proteína e carboidratos, sendo influenciados tanto pela dieta quanto por variáveis não nutricionais como produção, raça e estágio de lactação (DOSKA et al., 2012). Excesso de PB ou falta de energia fermentável aumentam o NUL (NOUSIAINEN, 2004), enquanto dietas adequadamente balanceadas promovem maior eficiência no uso do nitrogênio.

Assim, a composição do leite emerge da interação dinâmica entre metabolismo ruminal, metabolismo intermediário, fisiologia mamária, ambiente térmico, estado sanitário e genética. Os fatores não nutricionais estabelecem o potencial e as restrições de cada animal, enquanto os fatores nutricionais modulam a expressão desse potencial no curto prazo.

2.3 Ritmos sazonais e variação anual da composição do leite

A variação sazonal da composição do leite é um fenômeno amplamente reconhecido e com impacto direto sobre a qualidade industrial, o rendimento de derivados e a eficiência dos sistemas de produção (BERNABUCCI et al., 2015). Ao longo do ano, oscilações previsíveis nos teores de gordura, proteína, lactose e sólidos totais refletem tanto mudanças ambientais, quanto ajustes homeostáticos que acompanham variações metabólicas e hormonais do animal (NIU et al., 2014; BERNABUCCI et al., 2015). Por décadas, esse comportamento foi atribuído quase exclusivamente à nutrição e ao estresse

térmico (PALMQUIST et al., 1993), sob a premissa de que a flutuação anual seria resultado direto da qualidade da dieta, do manejo de pastagens e das condições climáticas. No entanto, evidências recentes demonstram que fatores extrínsecos não explicam integralmente o padrão cíclico observado, sugerindo a existência de mecanismos fisiológicos endógenos que modulam a lactação ao longo do ano.

Estudos em mamíferos mostram que a fisiologia reprodutiva, metabólica e termorregulatória é estruturada em torno de ritmos circanuais sincronizados pelo fotoperíodo, o qual atua via secreção de melatonina e modula a atividade do eixo hipotálamo-hipofisário (LINCOLN et al., 2006). Em bovinos, há evidências de que o fotoperíodo influencia diretamente a lactação por meio da regulação de prolactina e serotonina, hormônios envolvidos na lactogênese, no comportamento alimentar e na taxa de secreção de leite (CHEW et al., 1979; DAHL et al., 2000; PICCIONE et al., 2012). A alternância anual entre dias longos e curtos constitui, portanto, um sinal temporal robusto que ajusta processos metabólicos de forma antecipatória, auxiliando o animal a se preparar para condições previsíveis de maior ou menor demanda energética. Esse mecanismo é coerente com a lógica evolutiva que favorece maiores teores de sólidos nos meses mais frios, em função das maiores exigências energéticas do neonato nesses períodos (NASEM, 2021).

A aplicação de ferramentas da cronobiologia para o estudo da lactação permitiu aprofundar o entendimento desses fenômenos. Estudos conduzidos nos Estados Unidos utilizando análise rítmica do tipo Cosinor demonstraram que a gordura e a proteína do leite seguem um padrão anual regular, com valores máximos próximos ao solstício de inverno e valores mínimos próximos ao solstício de verão (SALFER et al., 2019; SALFER et al., 2020). A consistência desses picos e vales ao longo de anos sucessivos e em regiões distintas reforça que o fotoperíodo atua como sincronizador primário desses ritmos, mesmo diante de variações substanciais em manejo, dieta e clima. Como discutido por Salfer et al. (2019), se nutrição ou estresse térmico fossem os principais determinantes das mudanças anuais, esperar-se-ia alterações abruptas e não o padrão gradual e contínuo observado nos teores de sólidos.

A literatura também indica que calor e nutrição não explicam adequadamente o padrão anual. A influência do estresse térmico sobre o teor de gordura, por exemplo, é frequentemente controversa: embora reduza a produção total de leite, diversos estudos reportam aumento percentual da gordura durante períodos quentes, em razão de queda proporcionalmente maior do volume de leite do que da produção de gordura (RHOADS

et al., 2009; WHEELOCK et al., 2010; BAUMGARD et al., 2011; RUNGRUANG et al., 2014). Esse comportamento é oposto ao padrão sazonal típico de redução da gordura em meses de alta temperatura, reforçando que o calor, isoladamente, não é responsável pelo ciclo anual de sólidos. Da mesma forma, variações nutricionais abruptas tendem a gerar respostas igualmente abruptas, incompatíveis com o padrão lento e gradual observado ao longo do ano (PALMQUIST et al., 1993; SALFER et al., 2019).

Além disso, estudos de ritmos circadianos mostram que a síntese de leite apresenta oscilações diárias moduladas por horário de alimentação e estado metabólico (NIU et al., 2014), e que esses ritmos diários interagem com ritmos anuais de forma integrada. Essa interação sugere a existência de uma hierarquia temporal envolvendo relógios biológicos internos, cuja organização lembra a estrutura cronobiológica observada em outras espécies domésticas e selvagens (LINCOLN et al., 2006; PICCIONE et al., 2012).

Do ponto de vista metodológico, a ritmometria baseada no modelo Cosinor tornou-se ferramenta central para quantificar a ritmicidade anual da composição do leite. O modelo ajusta uma função harmônica aos dados temporais, permitindo estimar parâmetros biologicamente relevantes, como mesor (valor médio ajustado ao ritmo), amplitude (intensidade da oscilação) e acrofase (momento do pico máximo), conforme descrito por Bourdon et al. (1995) e sistematizado por Koukkar & Sothern (2006). Esses parâmetros possibilitam discriminar padrões rítmicos verdadeiros de flutuações aleatórias e têm sido aplicados com sucesso na caracterização dos ritmos anuais de gordura e proteína em rebanhos leiteiros de diferentes regiões dos Estados Unidos (SALFER et al., 2019; SALFER et al., 2020).

2.4 Estresse por calor em vacas leiteiras

As mudanças climáticas ao longo das últimas décadas resultaram em maiores períodos de estresse térmico ao longo do ano, desafiando a gestão da atividade pecuária e o mecanismo termorregulatório dos ruminantes (THORTON et al., 2021)

Já é bem relatado na literatura científica os efeitos negativos de altas temperaturas ambientais sobre índices reprodutivos e produtivos de vacas leiteiras (ROTH, 2020). Com o aquecimento global houve o agravamento do problema e maior preocupação pela busca de soluções, pois regiões em que não havia o desafio da produção de leite em altas temperaturas passaram a enfrenta-lo. O estresse térmico (HS) caracteriza-se pela combinação entre acúmulo excessivo de calor e perda da capacidade de dissipação

(BAGATH et al., 2019; GONZALEZ-RIVAS et al., 2020), conforme teoria do estresse proposta por Selye (1937).

É importante destacar que quanto maior a produção de leite, maior a susceptibilidade das vacas ao estresse térmico, portanto, à medida que se avança em produtividade por animal, aumenta-se o desafio térmico do animal (ROTH, 2020). O estresse térmico é um dos fatores de grande importância e impactam negativamente tanto o bem-estar dos animais, quanto o resultado econômico das fazendas produtoras de leite.

O impacto financeiro se deve, principalmente, a redução no teor de proteína e na produção total de leite. Pate et al. (2020) relataram prejuízos anuais superiores a 2 bilhões de dólares na bovinocultura devido aos efeitos do estresse por calor. Desse montante, cerca de 50% são atribuídos a perdas na bovinocultura de leite (ST.-PIERRE et al., 2003). Além das perdas em produção, altas temperaturas podem aumentar os custos com saúde e, em casos mais extremos, pode levar animais a óbito (DROVERS, 2011).

A redução no desempenho produtivo e reprodutivo de vacas leiteiras expostas ao estresse térmico é oriunda de mudanças fisiológicas e comportamentais (GONZALEZ-RIVAS et al., 2020). Como aspecto comportamental, destaca-se que animais em estresse térmico tendem a aumentar o tempo em pé, reduzir tempo de descanso e ruminação, além de reduzir o consumo de alimento para reduzir a produção de calor metabólico (BECKER et al., 2020).

De forma geral, é tido que a faixa de conforto térmico para vacas de leite europeias varia entre 16-25° C (DAS et al., 2016). Porém, estudos mostram que animais de alta produção, devido ao seu maior consumo alimentar, produzem mais calor metabólico e tem limites de conforto térmico mais baixos: 4-15° C (KLEIN et al., 2014). Na faixa de temperatura termoneutra vacas leiteiras mantém uma temperatura corporal entre 38,4 e 39,1°C (DAS et al., 2016). Temperaturas ambientais entre 20-25°C em clima temperado e 25-37°C em clima tropical, prejudicam a dissipação de calor pelas vacas e induzem HS, gerando sintomas como: elevação da temperatura superficial e retal, além de aumento da frequência respiratória e frequência cardíaca (DAS et al., 2016).

Comumente, o índice de temperatura-umidade (THI) é utilizado para avaliar HS em vacas de leite (BOHMANOVA et al., 2007; WILDRIDGE et al., 2018; AMMER et al., 2018). O THI é aplicável para avaliação do HS em vacas leiteiras em ambientes internos ou externos (HILL; WALL, 2015), diferentes climas e sistemas de produção (CARABAÑO et al., 2016; HERBUT; ANGRECKA, 2018).

Diferentes fórmulas são utilizadas para cálculo do THI, sendo dadas em função das temperaturas do bulbo seco (TBS em °C), úmido (TBU em °C), Temperatura do ar (T em °C), Ponto de orvalho (PO, e, °C) e umidade relativa (UR em %). Algumas dessas formas de cálculo são dadas por: THI = T + 0.36 × PO + 41,2 (YOUSEF, 1985); THI = (0,8 × T) + [UR × (T - 14,3)/100] + 46,3 (BUFFINGTON et al., 1983); THI = 0,72 (TBS + TBU) + 40,6 (KADZERE et al., 2002); THI = (1,8 × T + 32) – [(0,55 – 0,0055 × UR) × (1,8 × T – 26)] (LEGRAND et al., 2011).

Há consenso sobre o limite superior crítico para o THI. Tradicionalmente perdas significativas de produtividade de vacas de leite eram atribuídas a valores acima de 72 para THI (VITALI et al., 2009; MESGARAN et al., 2022). No entanto, estudos mais recentes mostraram que vacas de leite com alto potencial genético para produção de leite sofrem estresse térmico e tem perdas produtivas quando o THI ultrapassa (ZIMBELMAN et al., 2009; MESGARAN et al., 2022). Nesse contexto, o monitoramento do THI e de alguns parâmetros fisiológicos de vacas pode ser uma importante ferramenta para verificar a ocorrência de HS (HERBUT; ANGRECKA, 2018).

2.5 Respostas fisiológicas e comportamentais ao estresse por calor

Vacas leiteiras utilizam múltiplas adaptações fisiológicas para regular a temperatura corporal e manter a homeotermia. Os principais mecanismos agudos para a perda de calor são a perda de calor evaporativo através da transpiração e o aumento da taxa de respiração (LEMERLE; GODDARD, 1986; KADZERE et al., 2002), aumento no consumo de água (SILANIKOVE et al., 1998; BERNABUCCI et al., 2010), e redução no consumo de matéria seca (COWLEY et al., 2015; GAO et al., 2017) para reduzir a produção ruminal de calor. Quando a temperatura do hipotálamo está acima de valores críticos ativa-se mecanismos adicionais de perda de calor, como vasodilatação e sudorese (COLLIER et al., 2019).

Em relação a mudanças comportamentais, experimentos relataram aumento do tempo em pé e redução do tempo deitado, quando vacas estavam sujeitas a condições de HS (THI >68; COOK et al., 2007; ALLEN et al., 2015; NORDLUND et al., 2019; PATE et al., 2020).

Quando o THI ultrapassa os valores críticos de 68 (HEINICKE et al., 2018) e 72 (PINTO et al., 2020), há início de alterações fisiológicas (KADZERE et al., 2002), sendo que o limiar crítico varia conforme o parâmetro observado. O limite superior crítico é

menor, por exemplo, para mudanças na frequência respiratória do que para alterações na temperatura retal (PINTO et al., 2020).

Al-Qaisi et al. (2019) relataram um aumento de 1,2°C na temperatura retal e um aumento de 29 respirações/min na taxa de respiração para vacas em estresse por calor induzido comparado a vacas que não estavam em estresse por calor. Vários métodos podem ser utilizados para monitoramento da temperatura dos animais: temperatura vaginal (SAKATANI et al., 2012), termo-loggers de pele (LEE et al., 2016), temperatura ruminal (IPEMA et al., 2008), termografia infravermelha (PENG et al., 2019) e temperatura do leite (WEST, 2003).

Quando o aumento da frequência respiratória ocorre por períodos prolongados, há aumento da perda de CO2 e, consequente redução do poder de tamponamento do sangue, podendo acarretar alcalose respiratória (KADZERE et al., 2002; TAO et al., 2011; PATE et al., 2020). O HS induz o estresse oxidativo tecidual, com maiores níveis de espécies reativas de oxigênio (EROs) em animais em HS (PATE et al., 2020). Disfunção mitocondrial também foi observada em vacas leiteiras expostas ao HS.

A elevação da temperatura ambiental interfere negativamente sobre o centro de apetite do hipotálamo para induzir a redução do consumo de alimento (AMMER et al., 2018). Vacas em condições neutras de temperatura consomem de 12 a 15 refeições por dia, mas diminuem a frequência alimentar para 3 a 5 refeições por dia durante o estresse térmico (KADZERE et al., 2002). A redução da frequência alimentar acarreta necessidade de refeições maiores, podendo influenciar negativamente a saúde intestinal.

Menor ingestão de nutrientes está associado a aumento dos níveis plasmáticos de ácidos graxos não esterificados (AGNE; RHOADS et al., 2009), mas em vacas submetidas ao HS é comum observar menores níveis de AGNE no plasma (WHEELOCK et al., 2010; BAUMGARD; RHOADS, 2013). A insulina também é um potente hormônio antilipolítico (BAUMAN; VERNON, 1993) e o quadro de hiperinsulinemia no HS pode ser uma explicação para menor mobilização de triglicerídeos, quando comparado a vacas em balanço energético negativo. Foi demonstrado que o HS aumenta a lipase lipoprotéica (SAMMAD et al., 2020). Como a lipase lipoprotéica é a enzima que catalisa a hidrólise das lipoproteínas de densidade muito baixa sugere-se que há aumento do anabolismo de triglicerídeos.

Vacas em HS estão associadas a maior risco de lipidose hepática (BASIRICÒ et al., 2009) e, com isso, redução da secreção de albumina e da atividade de enzimas hepáticas (RONCHI et al., 1999).

A redução de consumo acarreta também em redução da mastigação, ruminação e, consequentemente, redução da produção e entrada de tamponantes no rúmen (SAMMAD et al., 2020). Além da redução da mastigação, há maior utilização de HCO3 para o sistema de tamponamento sanguíneo, reduzindo a quantidade de HCO3 disponível para sistema de tamponamento ruminal (KADZERE et al., 2002).

Associação entre hipertermia crônica e inapetência grave ou prolongada também é mencionada na literatura (KADZERE et al., 2002). Mudanças endócrinas como redução de somatotrofinas circulantes e aumento de IGF-II ajudam a entender a redução de consumo de animais em HS (MCGUIRE et al., 1991).

O HS está associado a mudanças no eixo hipotálamo-pituitária-adrenal (HPA), liberando glicocorticoides e aldosterona (VON BORELL, 2001). Maior liberação de aldosterona estimula a retenção de sódio e excreção de potássio pelo sistema renal. Dentre os glicocorticoides, destaca-se maior secreção de cortisol em vacas durante o evento de HS (ELVINGER et al., 2001). Durante o HS há maior produção de noradrenalina e epinefrina circulantes (STARKIE et al., 2005). Em última instância, essas alterações endócrinas reduzirão níveis de tiroxina (T4) e triiodotironina (T3) no plasma, reduzindo o metabolismo basal e, consequentemente, produção de calor (GAUGHAN, 2012).

É relatado na literatura aumento da concentração plasmática de hormônio antidiurético (ADH) em bovinos durante eventos de HS, podendo resultar em débito urinário (SAMMAD, 2020), podendo ser uma forma de compensação do organismo para as perdas de água na forma de sudorese. Aumento das concentrações de prolactina também foram observadas durante HS, que podem estar envolvidas no aumento do suprimento de água e eletrólitos (COLLIER et al., 1982).

Trabalhos com vacas em HS crônico demonstraram redução dos níveis plasmáticos de somatotropina (MCGUIRE et al., 1991; SUNDRUM, 2015) e de RNAm do IGF-1 no fígado (RHOADS et al., 2010), importantes hormônios para produção de leite.

O HS também altera o metabolismo de carboidratos e homeostase de glicose (WHEELOCK et al., 2010; RHOADS et al., 2007). Baumgard et al. (2011) também relataram mudanças no metabolismo pós absortivo de glicose em vacas submetidas HS.

Mesmo quando há ingestão semelhante de nutrientes, vacas em condições termo neutras parecem reduzir a utilização de glicose dependente da insulina, enquanto o grupo em estresse por calor mantém a utilização da glicose dependente da insulina, tanto em tecidos periféricos, quanto no fígado, aumentando a gliconeogênese durante o evento de

estresse por calor, indicando que a adaptação hepática à redução da ingestão de nutrientes, em termos de metabolismo de glicose, difere com variações na temperatura ambiental (WHEELOCK et al., 2010). Baumgard e Rhoads (2013) relataram que o aumento da concentração plasmática de prolactina poderia estar associado ao suporte da hiperinsulinemia, comumente observado em vacas em HS. Maior quantidade de transportadores de glicose independentes de insulina (GLUTs) foram observados in vitro para situação de HS, comparado a situação termoneutra (AHMED; BERRIDGE, 1998).

Maior secreção de glicocorticoides devido a mudanças no eixo hipotálamo-pituitária-adrenal (HPA), também estão associadas a mudanças no metabolismo de carboidratos (VON BORELL, 2001). Quando submetidas a HS pode haver redução da quantidade de receptores de GH em vacas de leite, que podem estar envolvidas na regulação da gliconeogênese (RHOADS et al., 2007). Qu et al. (2015) demonstraram aumento dos níveis de IGF-1 em vacas lactantes submetidas a HS, indicando aumento de processamento de triglicerídeos no fígado.

Em vacas submetidas a hipertermia ocorre a ativação do sistema nervoso autônomo, mediado por adrenalina e noradrenalina que acarreta o aumento frequência respiratória e cardíaca e do ritmo de recirculação de sangue com um fluxo secundário restrito de sangue e nutrientes para o trato gastrointestinal (TGI), consequentemente alterando o metabolismo celular de Ca e energia nos tecidos do TGI, reduzindo a capacidade adaptativa ao calor (HALL et al., 2001).

Estudos in vitro e in vivo já mostraram que o calor pode afetar a permeabilidade e função intestinal (LAMBERT et al., 2002; DOKLADNY et al., 2006) e Fontoura et al. (2022) demonstrou efeito similar em trabalho in vivo com vacas submetidas a HS. Maior fluxo sanguíneo periférico e menor disponibilidade de fluxo de sangue no TGI durante o HS (LAMBERT, et al., 2002) pode acarretar maior risco de hipóxia intestinal (HALL et al., 1999; HALL et al., 2001) e afrouxamento das junções estreitas intestinais e, assim, aumentar a passagem de LPS para o sangue (LAMBERT, et al., 2002; HALL et al., 2001). A inflamação induzida por LPS pode ativar a resposta imune, o que condiz com os achados que o estresse oxidativo, pode levar à inflamação e disfunção imunológica, ambos contribuindo para distúrbios metabólicos (DU et al., 2018; ZHU et al., 2019).

O HS acarreta maior permeabilidade do epitélio mamário de vacas em lactação, prejudicando a integridade do epitélio (WENG et al., 2018).

O estresse térmico afeta diretamente o metabolismo de vacas leiteiras. Cowley et al. (2015) relataram que o estresse térmico pode afetar o metabolismo de compostos

nitrogenados e alterar a partição do N em dietas de vacas leiteiras, com redução do teor de proteína do leite e aumento nos teores de nitrogênio ureico no leite.

Devido á redução dos níveis séricos de AGNE nas vacas em HS, há redução na disponibilidade de ácidos graxos voláteis (AGVs), fazendo com que glicose e AA sejam os substratos oxidativos mais disponíveis. Com isso é normal observar aumento nos níveis plasmáticos de nitrogênio ureico em vacas em HS (SHWARTZ et al., 2019). Marcadores de catabolismo muscular como 3-metil-histidina ou a creatina, encontram-se comumente elevados no plasma de vacas lactantes em HS (SCHNEIDER et al., 1988).

A concentração sanguínea de alanina, glicose, aspartato e glicina, associada à gliconeogênese, mostra-se significativamente aumentada sob HS (GUO et al., 2018).

Variação altamente significativa de hematócrito, hemoglobina, glicose plasmática, proteína total e albumina tem sido relatada para vacas avaliadas em diferentes temperaturas ambientais (SEJIAN et al., 2013).

As adipocinas, ou adipocitocinas, são citocinas e constituem em um hormônio peptídico normalmente produzido pelo tecido adiposo saudável e conhecido por sua ação sensibilizadora da insulina. Além da sensibilidade à insulina, essas moléculas podem influenciar uma variedade de processos fisiológicos, entre eles, o controle da ingestão alimentar, homeostase energética, angiogênese, proteção vascular, regulação da pressão e coagulação sanguínea (BOOTH et al., 2005). As adipocinas estão associadas com estresse oxidativo e processos inflamatórios (MCGOWN et al., 2014; MURRI et al., 2014).

Proteínas de choque térmico (HSPs) são as moléculas mias comumente relatadas e estudadas em quadros de hipertermia. Estão envolvidas na proteção celular e a HSP70 tem um papel de destaque nessa cadeia, sendo um bom preditor para HS (MIZZEN; WELCH, 1988; SAMMAD, 2020). Foi demonstrado que o HSP 70 está presente extracelularmente em ratos, humanos e bovinos (KRISTENSEN et al., 2004; FLESHNER E JOHNSON, 2005) e que os níveis plasmáticos de HSP 70 aumentam em resposta a uma variedade de fatores de estresse (JOHNSON et al., 2005; ANEJA et al., 2006).

A resposta ao estresse térmico é uma cascata altamente conservada de ativação proteica e de alteração da expressão genética (SONNA et al., 2002; COLLIER et al., 2006). A expressão genética desta rede de ativação está sob regulação do fator de transcrição de choque térmico (HSF; PIRKKALA et al., 2001; PAGE et al., 2006). A alta expressão de HSPs durante o estresse por calor está associado a citoproteção contra hipertermia, choque circulatório e isquemia cerebral (LEE et al., 2006).

Tradicionalmente, a resposta ao HS é considerada um fenômeno intracelular com pouca ou nenhuma associação com sinalização extracelular, porém, Collier et al. (2008) propôs que a resposta do HS está totalmente integrada com a resposta fisiológica ao estresse e deve ser considerada como um componente de uma rede genética sistêmica, incluindo células e tecidos.

A exposição celular à temperatura elevada induz uma série de anomalias na função celular (SONNA et al., 2002), que incluem: inibição geral da síntese proteica, defeitos na estrutura e função proteica, alterações morfológicas devido a rearranjos de citoesqueleto, mudanças no metabolismo, alterações na dinâmica da membrana celular e fluidez, e diminuição da proliferação celular. Essas anomalias invocam grandes mudanças na transcrição genética e na síntese proteica conhecida como resposta ao estresse por calor (LANKS, 1986; LINDQUIST, 1986). O tempo e o sucesso dessas alterações, em última análise, determinam a sobrevivência celular e aclimatação ou morte celular (COLLIER et al., 2008). Além disso, cada tecido ou tipo celular terá uma resposta e um nível de tolerância próprio ao HS (COLLIER ET AL., 2008).

A ativação do sistema de transcrição de choque térmico (HSF) inicia-se com temperaturas superiores a 35° C e, segundo Collier et al. (2008), as alterações na expressão genética incluem 1) ativação do sistema HSF; 2) aumento da expressão de proteínas do choque térmico (HSP) e redução da expressão e síntese de outras proteínas; 3) aumento da oxidação da glicose e aminoácidos e redução do metabolismo de ácidos graxos; 4) ativação endócrina do sistema da resposta ao estresse; e 5) ativação do sistema imunológico através de secreção extracelular de HSP. Se o estresse persistir, essas alterações na expressão genética levam a um estado fisiológico alterado chamado de aclimatação, um processo amplamente controlado pela expressão genética alterada em resposta aos sinais endócrinos, visando minimizar os impactos negativos do HS no organismo animal. O tempo para aclimatização varia, conforme o tecido ou célula, podendo ser de horas a semanas. Determinar a base para o metabolismo energético alterado durante o estresse térmico levará a oportunidades de melhor desempenho animal por meio de um manejo nutricional alterado (COLLIER et al., 2008).

Animais podem ter diferentes manifestações fisiológicas quando expostos ao HS, sendo que alguns indivíduos podem ter maior tolerância a esses eventos, indicando inclusive participação de fatores genéticos. Dessa forma, a identificação de vacas com fenótipos termotolerantes (AMAMOU et al., 2019), associado à utilização de técnicas

genômicas pode auxiliar no efetivo ganho genético para resistência ao HS (CARABAÑO et al., 2019).

Com todas as mudanças metabólicas, bioquímicas e fisiológicas que ocorrem durante HS, trabalhos estimam um aumento de 25-30% da energia necessária para mantença em vacas durante o HS (SAMMAD et al., 2020).

2.6 Estresse por calor e perdas produtivas

Devido as mudanças fisiológicas durante o HS, há a maior gasto energético, que deveria ser compensado com uma maior ingestão de MS, porém, como o consumo diminui, o HS impacta negativamente uma variedade de parâmetros produtivos, incluindo produção e composição do leite (PRAGNA et al., 2017), crescimento, reprodução e qualidade de carcaça (VITALI et al., 2009).

Wang et al. (2010) relataram que, devido à redução no consumo de alimentos e aumento na exigência de mantença, vacas em estresse térmico podem diminuir a disponibilidade de nutrientes para a produção de leite. A transformação da glicose em combustível preferencial nos quadros de HS é fator chave para comprometimento das respostas fisiológicas homeostáticas e para redução do potencial produtivo e reprodutivo das vacas em HS (SAMMAD et al., 2020).

Archer et al. (2013) relataram maior contagem de células somáticas no leite durante o verão, Smith et al. (2013) e Liu et al. (2017) relataram a redução do teor de gordura do leite de vacas Holandesas e Jersey submetidas ao estresse térmico e Bernabucci et al. (2015) verificaram que em situações de estresse térmico, poderia ocorrer redução no teor de proteína do leite, através da redução da concentração de caseína.

A redução na ingestão de matéria seca acarreta menor disponibilidade de nutrientes para os animais, incluindo os aminoácidos. Esse cenário é ainda mais crítico para dietas deficientes em AA, pois acarreta aumento do gasto energético para mobilização de AA de origem tecidual (PATE et al., 2020).

Historicamente, a redução no consumo de matéria seca (CMS), durante os eventos de estresse por calor, tem sido considerada o principal fator para redução da produção de leite (BEEDE; COLLIER, 1986; WEST, 1999). No entanto, alguns trabalhos relataram que a redução no CMS representa aproximadamente 35 a 50% da redução na produção de leite e que outras alterações fisiológicas e metabólicas mais crônicas também

influenciam diretamente perdas de produção (WHEELOCK et al., 2010; BAUMGARD et al., 2011, RHOADS et al., 2009).

Dentre as outras possíveis explicações para redução no desempenho de vacas leiteiras em estresse por calor, cita-se a redução da disponibilidade de aminoácidos precursores de proteína do leite na glândula mamária, devido ao aumento da utilização de AA para outros processos bioquímicos, como a gliconeogênese, síntese de proteínas de respostas da fase aguda do sistema imunológico e síntese de proteína de choque térmico (BERNABUCCI; CALAMARI, 1998; COLLIER et al., 2008; BAUMGARD; RHOADS, 2013; RÍUS, 2019). Além disso, alterações independentes no metabolismo pós-absortivo de glicose e lipídios estão associadas como causas adicionais das perdas de produtividade (WHEELOCK et al., 2010).

A hiperinsulinemia e falha na mobilização do tecido adiposo são apontadas como falhas de mecanismos poupadores de glicose, acarretando maiores gastos energéticos por vacas em HS e, consequentemente, menor energia disponível para lactação (WHEELOCK et al., 2010; RHOADS et al., 2009). Para suportar a produção de leite é necessário o aporte de glicose para produção de lactose, porém, as adaptações metabólicas para gerar menos calor metabólico, com destinação da glicose para outras funções pode ser uma das explicações para redução na produção de leite em vacas em HS (SAMMAD et al., 2020).

As perdas produtivas de vacas em diferentes estágios de lactação são relatadas em diferentes magnitudes durante o HS. Foi observado um declínio de 35% de produção para vacas no meio da lactação (RHOADS et al., 2009) e 14% para vacas no início da lactação (BERNABUCCI et al., 2010). Mecanismos adicionais precisam ser mais bem elucidados e podem auxiliar na compreensão das perdas de produção de leite em HS. Silanikove et al. (2009), por exemplo, mostrou que um derivado da b-caseína age como um ligante e se liga às células epiteliais mamárias, interrompendo os canais de potássio e, finalmente, reduzindo a síntese de leite em quadros de HS, por gerar incapacidade de utilização de precursores do leite oriundos do sangue.

De forma geral, a criticidade das respostas inflamatórias em vacas de leite, que é um evento comumente relatado em exposição ao HS (PATE et al., 2020), pode ser caracterizada por um aumento da concentração circulante de haptoglobina, que consiste em uma proteína de fase aguda positiva, e menor biossíntese de albumina, uma proteína de fase aguda negativa (BERTONI et al., 2008). Normalmente, os níveis de haptoglobina mostraram-se evidentemente mais elevados em vacas leiteiras não saudáveis do que em

animais saudáveis (STEFANSKA et al., 2018). Mesgaran et al. (2022), avaliando vacas em HS, observaram menores concentrações de haptoglobina quando utilizada suplementação com Zinco e MPR em comparação ao grupo sem suplementação. Tais achados indicam menor propensão à inflamação aguda e, consequentemente, melhor estado de saúde em vacas que suplementadas com MPR e Zinco, durante o período HS.

A inflamação e vacas de leite está diretamente ligada à capacidade produtiva, Bertoni et al. (2008) observaram que vacas com altos índices de inflamação produziram 20% menos leite durante o primeiro mês de lactação do que vacas com menores índices de inflamação.

Vacas em HS tendem a maior ativação do sistema imunológico e, consequentemente, maior resposta imune do sistema mamário (SALAMA et al., 2020), o que ajuda a explicar o aumento da contagem de células somáticas comumente observada em vacas lactantes em HS (ARCHER et al., 2013; HAGIYA et al., 2017).

Como consequência direta do HS, há aumento do estresse oxidativo no organismo, resultando em desequilíbrio de moléculas antioxidantes e oxidativas, como espécies reativas de oxigênio e peróxidos lipídicos (MESGARAN et al., 2021).

Oxilipídeos são importantes mediadores de regulação para equilíbrio das respostas pró e anti-inflamatória e qualquer alteração no metabolismo lipídico pode acarretar danos na função dos oxilipídeos (SORDILLO, 2018). Nesse contexto, o estresse oxidativo pode aumentar a produção de oxlipídios derivados do metabolismo de ácidos graxos ômega-6 da membrana celular e pode contribuir para processos inflamatórios (LOOR et al., 2013; MAVANGIRA; SORDILLO, 2018).

O malonaldeído (MDA) consiste em uma molécula de potencial citotóxicos, pois oxida DNA, proteínas e lipídios e estabelece ligações cruzadas entre grupos laterais AA e está associada a quadros de HS (BERNABUCCI et al., 2002). Nesse contexto, detecção de MDA pode ser utilizado como indicador de efeitos sistêmicos de estresse oxidativo persistente. O estresse oxidativo é um dos fatores que está diretamente relacionado com redução da capacidade imunológica durante o HS. Assim, a alteração dos metabólitos oxidativos em vacas influenciaria a produção de citocinas (SORDILLO; RAPHAEL, 2013).

Vacas em HS também estão mais propensas a problemas de saúde. Vacas leiteiras de transição de verão são mais propensas a desenvolver cetose subclínica ou clínica (GANTNER et al., 2016) e estão sob maior risco de fígado gorduroso (BASIRICÒ et al., 2009). A necessidade de utilização de dietas mais densas e a menor capacidade de

tamponamento ruminal aumenta as chances de acidose subclínica em vacas em HS. Eventos de HS também estão associados a maior incidência de patógenos ou seus vetores (CHIRICO et al., 1997) ou redução da capacidade defensiva de vacas (GIESECKE, 1985), aumentando a incidência de doenças patogênicas. Há relatos de hipocalcemia sérica em vacas de leite em HS, com consequência de aumento no risco de manifestação de doenças uterinas (MARTINEZ et al., 2012).

Problemas reprodutivos estão associados a vacas em HS (ROTH, 2020). O HS pode gerar consequências negativas de forma direta e indireta para o sistema reprodutivo (SAMMAD et al., 2020). Os efeitos diretos originam-se do comprometimento do ambiente folicular dos ovários e de danos ao desenvolvimento dos oócitos (PAES et al., 2016), ao passo que os efeitos indiretos estão relacionados a influência do HS sobre aspectos de nutrição e de produção de proteínas e hormônios relacionados ao sistema reprodutivo (PAES et al., 2016; ROTH, 2020).

A redução do consumo e mudanças de particionamento de nutrientes é a primeira causa para redução da disponibilidade de nutrientes para reprodução. São muitos os aspectos fisiológicos envolvidos no HS que prejudicam a reprodução de vacas de leite, dos quais destaca-se: maior dificuldade em identificação de estro (menor intensidade e duração) (HEIN; ALLRICH, 1992; HANSEN, 2019; ROTH, 2020); redução na viabilidade do sêmen (no caso de machos) e no desenvolvimento do oócito (HANSEN, 2019; ROTH, 2020); aumento da mortalidade embrionária, especialmente até sete dias; alteração na dinâmica de crescimento folicular; alteração no perfil hormonal com menor nível de estradiol no estro, menor secreção de LH (GILAD et al., 1993) e maior de FSH (HANSEN, 2019; ROTH, 2020). Elevação do nível plasmático de cortisol é relatado em HS e está envolvido em prejuízos reprodutivos (ELVINGER et al., 1992). Níveis altos de corticotrofina (ACTH) estão associados ao bloqueio de manifestação de estradiol e, consequentemente, do estro e aumento da secreção de corticosteroides pode inibir LH (GILAD et al., 1993).

2.7 Estresse por calor e redução na síntese de proteína do leite

O HS afeta negativamente a síntese de proteína do leite, que consiste em importante parâmetro de qualidade do produto (RHOADS et al., 2009; WHEELOCK et al., 2010; GUO et al., 2021). Essa redução na síntese de proteína é, na maioria dos trabalhos, atribuída a redução do consumo de alimentos (FUQUAY, 1981; BEEDE;

COLLIER, 1986; WEST, 1999; PATE et al., 2020). Porém, estudos com alimentação por pares já demonstraram que a redução de consumo explica apenas parte das reduções na produção de proteína (WHEELOCK et al., 2010; BAUMGARD et al., 2011. BAUMGARD; RHOADS, 2013; GAO et al., 2017).

O HS, além de reduzir o teor de proteína, altera o perfil de AA do leite, sugerindo maior mobilização de AA para resposta imune e gliconeogênese, em detrimento da síntese proteica do leite em vacas sob HS (GUO et al., 2018; GUO et al., 2021), explicado principalmente pela redução de AGNE circulante.

O elevado catabolismo proteico fora da glândula mamária e o consumo de aminoácidos durante para outras funções metabólicas durante HS têm sido apontado como principal justificativa para reduções na produção de proteínas e leite (GAO et al., 2017). A redução da capacidade de absorção intestinal de AA durante o HS, com consequente menor disponibilidade de AA para glândula mamária também é uma possível hipótese reduções na produção de proteínas e leite (MCGUIRE et al., 1989) .

Nardone et al. (2006) encontraram redução nos percentuais de caseína, lactalbumina, imunoglobulina G, imunoglobulina A durante o HS. Cerca de 80% das alterações encontradas foram associadas à perda de produtividade e 20% a problemas de saúde que podem ser devidos à ruptura do mecanismo interno de homeostase de vacas leiteiras (NARDONE et al., 2006). A redução dos níveis de proteína do leite das vacas em HS (RHOADS et al., 2009) mostra que a redução de síntese de alfa e b-caseína é a mais suscetível nessas condições (BERNABUCCI et al., 2002). Concentração plasmáticas de Lis menores foram encontradas para vacas em HS, quando comparadas a vacas termo neutras (GUO et al., 2018).

Estudos sugerem que HS causa estresse oxidativo (EO) com altos níveis de espécies reativas de oxigênio e nitrogênio nos tecidos, que pode resultar em indução de apoptose celular, reduzindo o número de células epiteliais mamárias, além de desregulação endócrinas, que em última instância podem atuar na redução de produção de proteína no leite (GUO et al., 2021).

Em resumo, danos diretos ao tecido mamário, redução da capacidade de funcionamento da glândula mamária, mudanças no metabolismo energético, mudanças na concentração de hormônios envolvidos na lactação e aumento do catabolismo proteico são os principais mecanismos para explicar as perdas de produção de leite de vacas durante o HS (SAMMAD et al., 2020).

2.8 Mitigação dos efeitos negativos do estresse por calor em vacas leiteiras

Há várias propostas para minimizar os efeitos negativos do estresse por calor em vacas leiteiras: pulverização, ventilação, sombreamento (BECKER et al., 2020). Embora esses manejos sejam comumente adotados, o estresse por calor ainda causa problemas econômicos significativos para os produtores de leite (PATE et al., 2020) e outras iniciativas devem ser utilizadas para tentar minimizar o problema, tais como as estratégias nutricionais.

É importante ressaltar que a primeira ação para redução de impactos negativos do HS em vacas de leite deve ser o resfriamento ambiental, com redução da temperatura nos galpões ou resfriamento diretamente dos animais, sendo tais manejos as estratégias de maior retorno (HANSEN, 2019; ROTH, 2020). Após realizado ajustes no ambiente, estratégias nutricionais e de biotecnologias da reprodução podem ser utilizadas para obter melhores resultados em situação de HS.

O consumo deficitário de todos os nutrientes, em especial: aminoácidos, minerais e vitaminas que participam de moléculas antioxidantes pode reduzir a capacidade do organismo de síntese dessas moléculas e agravar o quadro de HS (por exemplo: metionina, vitamina E, selênio, cobre, zinco). O menor consumo desses nutrientes pode ocorrer principalmente por redução no consumo de matéria seca como consequência de resposta fisiológica das vacas em tentar reduzir o calor metabólico (DU et al., 2018; ZHU et al., 2019).

Para compensar a redução do CMS em vacas em HS, o aumento da densidade energética da ração é uma importante ferramenta para garantir o adequado suprimento nutricional (DRACKLEY et al., 2013). Apesar de ser importante estratégia para minimização de impactos negativos do HS é necessário cuidado extra, pois, por alterações fisiológicas já mencionadas, vacas em HS podem estar mais susceptíveis a acidose. O adensamento energético é uma estratégia importante, pois além da redução no CMS, HS acarreta alterações do metabolismo energético, respostas inflamatórias e disfunção imunológica, aumentando as demandas energéticas dos animais. (DU et al., 2018; ZHU et al., 2019).

Devido ao aumento da utilização de AA limitantes da produção de leite para outras funções metabólicas, que não a síntese de proteína pela glândula mamária, a adequação do fornecimento de proteína não degradável no rúmen (PNDR), principalmente com a utilização de AA protegidos da degradação ruminal, é uma alternativa recorrentemente

proposta em diversos trabalhos para minimizar efeitos negativos da redução do CMS. Essa alternativa é capaz de auxiliar a produção de leite e a concentração de proteínas e gordura no leite, ao mesmo tempo em que melhora as respostas às condições de HS (OSORIO et al., 2013; ZHOU et al., 2016; BATISTEL et al., 2017). A adequada suplementação de AA também terá efeito na redução da produção de calor oriunda da mobilização de proteína corporal (PATE et al., 2020), além de AA como Met ser constituinte de diversas moléculas atuantes na atenuação dos impactos negativos do HS, como glutationa peroxidase, por exemplo.

Maior suplementação de minerais como sódio e potássio também são importantes, devido às perdas por sudorese (SAMMAD et al., 2020). Microminerais como Mn, Zn, Mo, P, e Se foram capazes de promover melhorias no estado fisiológico de vacas leiteiras em HS (BICALHO et al., 2014). Vitaminas do complexo B, ácido ascórbico, vitamina E (tocoferol), niacina protegida da degradação ruminal e ácido nicotínico também demonstraram potencial de melhorar o estado fisiológico de vacas leiteiras em HS (DI CONSTANZO, 1997).

Inclusão de ionóforos e monensinas demonstrou potencial de melhoria sobre o metabolismo energético e parâmetros produtivos de bovinos submetidos ao HS (BAUMGARD; RHOADS, 2011; BARRERAS et al., 2013). A inclusão de bicarbonatos na dieta também demonstraram potencial de auxiliar na manutenção do pH ruminal e reposição do HCO3 desviado para tamponamento sanguíneo (SAMMAD et al., 2020). Utilização de dietas aniônicas também pode auxiliar para minimizar os impactos do HS (WILDMAN et al., 2007). Uso de niacina mostrou potencial de melhorar o metabolismo em vacas leiteiras em HS (DI COSTANZO et al., 1998). Utilização de óleo de palma na dieta aumentou o consumo alimentar e reduziu os sinais de HS (MELO et al., 2018).

O uso de tiazolidinedionas (TZD), também conhecidas como glitazonas, mostraram potencial de aumentar a produção de proteínas do choque térmico (TANIGUCHI, 2006), melhorar utilização de glicose (SCHOENBERG; OVERTON, 2011) e o metabolismo energético (RANGANATHAN et al., 2006), podendo ser uma alternativa útil ao HS. Suplementação com cromo também está sendo demonstrada para melhorar o metabolismo energético e a produção de vacas em HS e uma das expicações é a participação desse mineral em enzimas antixoidantes (BIN-JUMAH et al., 2020).

Para redução dos prejuízos reprodutivos causados por HS, uma série de biotecnologias reprodutivas vem sendo estudadas e podem ser adotadas para aumentar taxas reprodutivas no verão, tais como: utilização de inseminação artificial em tempo fixo

para reduzir a necessidade de identificação de estro, tratamentos hormonais como a utilização de análogos mais ativos de mais ativos do GnRH, utilização de implantes de progesterona, aplicações de HCG, dentre outras (HANSEN, 2019; ROTH, 2020). biotecnologias apresentadas devem ser ajuste fino do sistema, sendo recomendadas apenas de forma complementar ao resfriamento ambiental ou dos animais. Portanto, é importante ressaltar que a redução da temperatura nos galpões ou diretamente dos animais, deve ser o primeiro passo para obtenção de mehores índices reprodutios de vacas em HS (HANSEN, 2019; ROTH, 2020).

Outra estratégia de longo prazo para aumentar a tolerância de vacas leiteiras ao estresse térmico é a seleção genética, pois há herdabilidade na raça holandesa de características como: capacidade de manutenção da temperatura corporal mais baixa em situações de estresse térmico e capacidade de manutenção da produção de leite em estado de estresse (HANSEN, 2019; CARABAÑO et al., 2019). Para viabilização do melhoramento genético deve-se promover a sistematização de registros fenotípicos e genotípicos, a fim de possibilitar a identificação de animais tolerantes ao calor em raças de alta produção (AMAMOU et al., 2019).

É possível a inserção de novos genes nas raças produtoras de leite para garantir melhor adaptação em situações de elevadas temperaturas (HANSEN, 2019). Para tal seria necessário a abordagem baseada em diferenças nas características anatômicas e morfológicas, que explicam parcialmente as diferenças de tolerância ao calor entre espécies e raças (WEST, 2003; DIKMEN; HANSEN, 2009; HANSEN, 2019).

2.9 Utilização de metionina como estratégia para mitigação de efeitos negativos do HS

A metionina é conhecida por ser um AA essencial que auxilia na melhoria do metabolismo e a saúde de vacas leiteiras (BATISTEL et al., 2017; MESGARAN et al., 2022). A Met tem funções importantes no organismo além da síntese proteica, tais como: metilação de DNA, regulação da tradução e síntese de outras moléculas, parte de moléculas importantes do sistema imunológico e do metabolismo antioxidante (BATISTEL et al., 2017).

Tradicionalmente, há dois métodos comerciais mais utilizados para proteção de Met da degradação ruminal. Um desses métodos é proteção por etil-celulose que visa a liberação do AA por abrasão do revestimento ao longo do trânsito intestinal, geralmente

resultando em um fluxo mais lento de Met e menores concentrações séricas do AA no plasma de vacas (BLUM et al., 1999; SUDEKUM et al., 2004). O outro método mais comumente encontrado é o revestimento por co-vinyl-polimero (MET-cop), que possui sensibilidade a menores valores de pH, resultando em rápida liberação de MET no abomaso, resultando em maiores concentrações séricas de Met (BLUM et al., 1999; SUDEKUM et al., 2004). Uma meta análise recente, porém, não encontrou correlação entre concentrações plasmáticas de Met no sangue e o desempenho de vacas (PATTON et al., 2022), tendo encontrado efeitos benéficos no desempenho para os dois métodos de revestimento.

Embora a utilização de metionina protegida da degradação ruminal (MPR) para minimização de impactos negativos do período de transição seja bem conhecida, poucos trabalhos estudaram a utilização desse ingrediente para redução de impactos do estresse por calor (PATE et al., 2020). Osório et al. (2013) observaram maior produção de proteína do leite em vacas alimentadas com metionina protegida da degradação ruminal (MPR) e Toledo et al. (2021) observaram maior produção de leite para vacas em HS alimentadas com MPR. Pate et al. (2020) estudaram a inclusão de 1,05 g de metionina protegida da degradação ruminal (MPR; Smartamine M; Adisseo Inc., Antony, França) por Kg de matéria seca da dieta sobre o desempenho e atividade imunológica de vacas holandesas em estresse por calor e não observaram diferenças nos parâmetros fisiológicos e nem na produção de leite e eficiência alimentar entre o grupo que recebeu MPR e o controle, porém, houve incremento de proteína do leite para o grupo que recebeu MPR, comparado ao controle (PATE et al., 2020). Entretanto, os resultados de suplementação com MPR são comumente inconsistentes, principalmente para respostas em produção de leite (MESGARAN et al., 2021; ZANTON et al., 2014).

Alguns estudos observaram aumento de gordura do leite com suplementação de MPR, quando comparado a grupos sem suplementação (MESGARAN et al., 2021; PATE et al., 2020). Alguns mecanismos foram propostos para explicar aumento de gordura do leite de vacas em estresse por calor suplementadas com MPR, dentre eles, destacam-se: 1) alívio do balanço energético negativo como consequência da melhoria do metabolismo e sistema imunológico do animal (BATISTEL et al., 2017). 2) Suplementação com Met pode induzir a condensação de triacilglicerol em VLDL no fígado, devido a maior síntese de apolipoproteína B-100 e, com isso, elevando o fluxo de ácidos graxos para síntese gordura do leite na glândula mamária (BAUCHART ET AL., 1998; OSORIO et al., 2013), porém não á uma consistência na literatura sobre o papel da Met formação de

VLDL no fígado (CHANDLER; WHITE, 2017). 3) Outro mecanismo poderia ser que um correto balanço de aminoácidos essenciais, principalmente Met, possa auxiliar na síntese mamária ácido graxos de novo, pelo aumento da expressão mRNA de enzimas lipogênicas (LI et al., 2016; BATISTEL et al., 2017). Patton (2010) observou que vacas com alimentação deficiente em Met produziram maiores teores de gordura no leite quando suplementadas com MPR. Embora comumente observado e postulado alguns mecanismos, o impacto da suplementação com MPR na regulação da síntese de ácidos graxos na glândula mamária precisa ser mais bem elucidado (MESGARAN et al., 2021).

Maiores concentrações de caseína e proteína no leite de vacas em estresse por calor suplementadas por MPR são comumente relatados (PATE et al., 2020) e um mecanismo para explicar essa maior síntese de proteína pode ser devido ao maior aporte de Met na composição da proteína metabolizável (SCHWAB; BRODERICK, 2017), além do efeito da suplementação de Met nas vias de sinalização da síntese proteica (via mTOR) no tecido epitelial mamário (NAN et al., 2014; LI et al., 2016). O aumento da síntese proteica do leite pode estar relacionado à ligação entre Met e AAs de cadeia ramificada no que diz respeito à regulação da biossíntese de proteína do leite (DONG et al., 2018).

Além das funções diretamente ligadas à produção, a metionina participa da composição de moléculas do sistema antioxidante, tal como a glutationa (GSH) que é um antioxidante bem conhecido e atua diretamente na manutenção e regulação do equilíbrio redox nas células animais (AQUILANO et al., 2014). A síntese de glutationa ocorre principalmente no fígado e tem a metionina diretamente envolvida nesse processo (MARTINOV et al., 2010; ZHOU et al., 2016). A metionina também participa da absorção de zinco (PREDIERI et al., 2005) e atua como agente lipotrópico para prevenir o acúmulo excessivo de gordura no fígado (ZHOU et al., 2016).

Trabalhos já demonstraram relação entre a suplementação com Met e redução de MDA em vacas submetidas ao HS (MESGARAN et al., 2021). Indicando potencial de utilização da Met para redução da produção de espécies reativas de oxigênio em vacas leiteiras, pois menor presença de MDA indica menor nível de peroxidação lipídica e melhor capacidade antioxidante de vacas em HS (MESGARAN et al., 2021). Aumento do fluxo de Met no duodeno em ovelhas resultou em redução dos índices plasmáticos de MDA (MAVROMMATIS et al., 2021).

Mesgaran et al. (2021) observaram ainda efeito positivo da suplementação de um composto com Zinco e Metionina sobre o desempenho de vacas leiteiras em HS, pela otimização do turnover proteico, conferindo melhor eficiência.

Relações de suplementação com MPR e redução de CCS em vacas leiteiras em HS foi observado (MESGARAN et al., 2021; PATE et al., 2020; SOBHANIRAD et al., 2010) e pode estar associado a melhoria de integridade epitelial da glândula mamária e melhor função imunológica desses animais (MESGARAN et al., 2021). De forma geral, vacas saudáveis quando expostas ao HS, apresentam melhor padrão metabólico menos inflamações agudas (HORST et al.; 2021).

Já foi demonstrado por experimento in vitro relação da Met com redução na expressão de genes da imunidade inata, como IL-1B e IL−6, possivelmente relacionados a redução da inflamação celular (LOPREIATO et al., 2019).

Suplementação com MPR também pode afetar a concentração de cálcio circulante. Wang e Beede (2010) observaram que o aumento da ingestão proteica durante o período seco, reduziu incidência de hipocalcemia pós parto em vacas da raça Jersey. A redução dos casos de hipocalcemia pode estar associada a melhoria do sistema imunológico como um todo em vacas com uma adequada suplementação de aminoácidos (HORST et al., 2021). Moraes et al. (2021) em trabalho com codornas poedeiras em HS, examinaram a relação entre suplementação com Met dietética e desempenho, localização e expressão dos canais transportadores de cálcio epitelial dos sistemas digestivo e reprodutivo. Os autores identificaram redução do efeito negativo do HS quando as aves receberam 120% de Met em relação à exigência.

SEGUNDA PARTE

Artigo 1 - Redigido conforme norma do periódico científico - Versão preliminar	
Título do artigo:	Annual rhythms of milk composition in dairy cattle in Brazil
Autores:	Caio Rodrigues Monteiro João Pedro Andrade Rezende Victor Augusto de Oliveira Isaac James Salfer Marina de Arruda Camargo Danés
Periódico:	Journal of Dairy Science
ISSN	1525-3198

ARTIGO 1 – Annual rhythms of milk composition in dairy cattle in Brazil

Running Head: Annual rhythms of milk composition in Brazil

Caio R. Monteiro[1], João Pedro A. Rezende[1], Victor Augusto de Oliveira[1], Isaac J. Salfer[2], and Marina C. A. Danés*[1]

[1]Department of Animal Science, Federal University of Lavras, Lavras, MG, 37200-900, Brazil

[2]Department of Animal Science, University of Minnesota, St. Paul, MN, 55108, USA

*****Corresponding author: Marina de Arruda Camargo Danes

E-mail: marina.danes@ufla.br; Phone: (+55) 35-99960-3101

Annual rhythms of milk composition in dairy cattle in Brazil.

Interpretative Summary: This study used large national and breed-association databases to show that milk fat, protein, and, to a lesser extent, somatic cell count in Brazilian dairy cows follow predictable annual rhythms at market, herd, and cow levels. Seasonal cosinor models explained most of the within-year variation in fat and protein, whereas milk and feed prices added little extra predictive power, indicating that biological and climatic drivers rather than short-term economics underlie these patterns. The results highlight consistent geographic differences in the timing and magnitude of these rhythms and reveal substantial between-herd and between-cow variation in seasonal responses, information that can be used to improve processing planning, farm management, and future genetic evaluations for seasonal resilience.

Abstract

This study aimed to characterize annual rhythms in milk composition and somatic cell count (SCC) in Brazilian dairy systems at market, herd, and cow levels, and to evaluate the relative contribution of seasonal versus economic drivers. Monthly bulk-tank fat, protein, and SCC data (January 2013–December 2023) from 6 major dairy states (792 state-month observations) were analyzed using 12-mo cosinor mixed models with state and year effects. Additional linear mixed models combined seasonal terms with state- and national-level prices of milk, corn, and soybean. Test-day records from the Girolando milk-recording program (10,987 observations from 718 cows in 41 herds across 3 states) were used to fit herd- and cow-level cosinor models and a set of validation mixed models. At the national level, inclusion of cosinor terms reduced residual sums of squares by ≈90% for fat and ≈81% for protein compared with models including only state and year, and ≈34% for SCC, with clear geographic gradients in mesor, amplitude, and acrophase.

Economic variables explained considerably less variation, and adding them to cosinor models produced only small gains in R^2. At the herd level, 65.9% of herds were classified as rhythmic for protein, 34.1% for milk yield, 31.7% for fat, and 22.0% for SCC. At the cow level, significant annual rhythms were detected in 20.5% of cows for fat, 46.2% for protein, 22.2% for SCC, and 51.6% for milk yield. These results demonstrate robust circannual patterns in milk composition that are conserved across organizational levels and are largely independent of short-term economic variation.

bulk-tank data, cosinor analysis, seasonal variation

Introduction

Seasonal variation influences milk composition and quality, with direct consequences for processing efficiency, product standardization, and farm profitability (Bernabucci et al., 2015). These periodic changes reflect not only environmental fluctuations but also physiological adjustments related to energy homeostasis and metabolic stress, leading to variation in milk fat, protein, and somatic cell count (SCC; Niu et al., 2014; Bernabucci et al., 2015).

Growing evidence indicates that, beyond immediate environmental effects, bovine lactation is modulated by endogenous biological rhythms similar to those described in other mammals (Lincoln et al., 2006). These rhythms are synchronized primarily by photoperiod, acting on the hypothalamic–pituitary axis via melatonin secretion, and in cattle have been linked to changes in hormones such as prolactin and serotonin, which are closely related to lactogenesis and mammary metabolism (Chew et al., 1979; Dahl et al., 2000; Piccione et al., 2012). Several studies in the Northern Hemisphere have identified consistent annual rhythms in milk yield and composition of

dairy cows (Salfer et al., 2019, 2020). The amplitude and phase of these rhythms vary with latitude and are more pronounced in regions with greater annual variation in day length, reinforcing their biological basis (Salfer et al., 2020). In addition, circadian and shorter-term rhythms in milk synthesis can be modulated by nutritional factors and feeding time, highlighting the integration between metabolic and chronobiological regulation (Niu et al., 2014).

The concept of physiological rhythmicity in cattle is supported by chronobiological theory, and its quantification can be achieved using cosinor-based rhythmometry, which yields biologically interpretable parameters such as mesor (rhythm-adjusted mean), amplitude, and acrophase (timing of the peak; Bourdon et al., 1995; Koukkari and Sothern, 2007). Such models have been successfully applied to describe annual rhythms in milk composition at large scale in the United States (Bourdon et al., 1995; Salfer et al., 2019). However, despite advances in temperate regions, few studies have evaluated these patterns under tropical and subtropical conditions. Brazil, one of the largest milk-producing countries, encompasses a wide diversity of production systems and climatic zones, imposing heterogeneous environmental and nutritional pressures. Although national programs such as the Brazilian National Milk Quality Program provide extensive data on milk composition, these databases have not yet been systematically explored to identify and quantify seasonal rhythms across multiple levels of organization. Furthermore, the interaction between biological seasonality and economic drivers, such as feed costs and milk prices, remains poorly understood.

Therefore, the objective of this study was to characterize annual rhythms in milk composition at multiple hierarchical levels: national, herd, and individual cow, and to evaluate the consistency of seasonal patterns among Brazilian regions. We hypothesized that robust annual rhythms would be detectable in milk composition, but with substantial

regional differences in amplitude and phase, reflecting the climatic, nutritional, and management diversity of Brazilian dairy systems.

Materials and methods

Cosinor analysis of Brazilian milk composition market data

Milk composition data from January 2013 through December 2023 were obtained from the Brazilian Ministry of Agriculture and Livestock (MAPA, Brasília, Brazil). The dataset consisted of monthly bulk tank milk samples analyzed by accredited laboratories within the Brazilian National Milk Quality Program. Concentrations of milk fat and protein (%) and somatic cell count were determined primarily by mid-infrared spectroscopy as part of routine payment-for-quality testing. The final national dataset comprised a total of 792 monthly state-level observations (6 states × 11 years × 12 months).

Data were aggregated by state (federal unit), which served as the geographic unit for statistical analysis. The dataset included the following states: Goiás (GO), Minas Gerais (MG), Paraná (PR), Rio Grande do Sul (RS), Santa Catarina (SC), and São Paulo (SP), which together represent the majority of Brazilian milk production and encompass a broad range of climatic and production conditions.

All statistical analyses were performed in R (version 4.5.1; R Core Team, Vienna, Austria). Monthly milk fat, protein, and somatic cell count were fit to the linear form of a 12-month cosine function following Bourdon et al. (1995), using mixed-effects regression as described by Niu et al. (2014).

We evaluated AR(1) correlation structures to account for temporal autocorrelation. Temporal autocorrelation was screened on the residual autocorrelation function (ACF) of the lmer fits; when the absolute ACF at lags 1–3 exceeded 0.2, models

were re-fitted with an AR(1) residual structure using nlme::lme. Otherwise, an independent residual structure was retained. The final model specification was:

$$Y_{\text{ijt}} = \beta_0 + \beta_{\text{state(i)}} + \beta_{\cos} \cos\left(\frac{2\pi t}{12}\right) + \beta_{\sin} \sin\left(\frac{2\pi t}{12}\right) + \beta_{\text{state(i):cos}} \cos\left(\frac{2\pi t}{12}\right) + \beta_{\text{state(i):sin}} \sin\left(\frac{2\pi t}{12}\right) + u_{\text{year(j)}} + \varepsilon_{ijt},$$

where Y_{ijt} is milk composition variable for state *i*, year *j*, and month *t*;

β_0 is overall intercept (mesor);

$\beta_{\text{state(i)}}$ is fixed effect of state *i*;

$\beta_{\cos}$ and $\beta_{\sin}$ are fixed coefficients for cosine and sine terms;

$\beta_{\text{state(i):cos}}$ and $\beta_{\text{state(i):sin}}$ are interaction terms between state and seasonal components;

$u_{\text{year(j)}} \sim N\left(0, \sigma^2_{\text{year}}\right)$ is the random intercept for year *j* to account for interannual variation; and

$\varepsilon_{\text{ijt}} \sim N(0, \sigma^2)$ is the residual error.

The fit of the 12-mo rhythm was evaluated by a zero-amplitude test, which employs a likelihood ratio test to compare the full model (including cosine and sine terms) with a reduced model excluding them (Koukkari and Sothern, 2007). The mesor (mean level), amplitude, and acrophase (time of peak) were estimated for each state according to Bourdon et al. (1995).

Brazilian milk composition model comparison: seasonal and economic predictors of milk composition

To evaluate the relative contribution of seasonal and economic factors to the variation in milk composition, a series of linear mixed-effects models were fitted for milk fat and protein concentrations using monthly bulk-tank data from January 2013 through

December 2023. The dependent variables were the monthly averages of fat (%) and protein (%) for each major dairy-producing state in Brazil.

Seasonal variation was modeled using a cosinor function with a 12-mo period, including the terms $\cos(2\pi t/12)$ and $\sin(2\pi t/12)$ to represent the cyclic annual oscillation in milk composition. All models included state (federal unit) as a fixed effect to account for regional differences.

Monthly state-level economic data were collected for the same period (2013-2023), including: (1) average milk price (state and national), and (2) monthly prices of corn and soybean (BRL/kg).

A total of 15 models were tested for each response variable, starting with the cosinor-only model and progressively including combinations of the economic predictors. Each model was defined as:

$$Y_{ijt} = \beta_0 + \beta_{\text{state(i)}} + \sum_{k=1}^{p} \beta_k X_{k,ijt} + u_{\text{year(j)}} + \varepsilon_{ijt},$$

where Y_{ijt} is the milk fat or protein concentration for state *i*, year *j*, and month *t*;

β_0 is the overall intercept;

$\beta_{\text{state(i)}}$ is fixed effect of state *i*;

β_k are the fixed coefficients associated with the economic and seasonal predictors $X_{k,ijt}$;

$u_{\text{year(j)}} \sim N\left(0, \sigma^2_{\text{year}}\right)$ is the random intercept for year *j*; and

$\varepsilon_{ijt} \sim N(0, \sigma^2)$ is the residual error.

Models were fitted using restricted maximum likelihood with the lmer() function from lme4 package in R version 4.5.1. Temporal autocorrelation was screened using residual ACF from the lmer fits; if |ACF| at lags 1–3 exceeded 0.2, the model was re-estimated with an AR(1) residual structure via nlme::lme; otherwise, an independent residual structure was retained.

Model performance was evaluated using Akaike information criterion (AIC), Bayesian information criterion (BIC), root mean square error (RMSE), and marginal R^2 calculated via Nakagawa's method. For state-specific analyses, all models were fitted independently for each state using the same mixed-effects structure to assess within-state predictor performance.

Cosinor analysis of herd and individual cow milk composition

Milk composition data were obtained from the database of the Brazilian Association of Girolando Breeders (Uberaba, Minas Gerais, Brazil), which compiles routine test-day records from commercial dairy herds enrolled in the official Girolando milk-recording program. Milk samples were analyzed for fat and protein contents, and somatic cell count (SCC) using mid-infrared spectroscopy in accredited laboratories certified for payment-for-quality testing.

The initial dataset contained 124,844 test-day records from 39,898 cows in 519 herds between January 2013 and December 2023. Records were excluded when milk fat was <2.5% or >5.5%, protein <2.5% or >5.5%, or when month or state information was missing, and only cows with ≥11 valid monthly test-day observations were retained. Because only 3 major dairy-producing states were consistently represented in the Girolando database, the dataset was restricted to Minas Gerais, São Paulo, and Paraná. At the herd level, eligibility required contributing ≥3 cows meeting the ≥11-test-day criterion and having records in ≥6 distinct months during the study period. After applying all record-, cow-, and herd-level filters, the final dataset used for cosinor modeling consisted of 10,987 observations from 718 cows belonging to 41 herds.

All analyses were performed using R version 4.5.1. Cosinor models were based on the linear form of a cosine function with a 12-month period (Bourdon et al., 1995).

For herd and cow-level models exhibiting significant temporal autocorrelation, AR(1) covariance structures were applied to account for serial correlation in repeated monthly measures. Rhythm parameters (mesor, amplitude, acrophase) were derived from model coefficients. The significance of the annual rhythm was assessed via a zero-amplitude F-test (Koukkari and Sothern, 2007).

Herd-levels analysis

To identify which herds display significant annual rhythms in milk composition and to characterize their herd-specific seasonal parameters (amplitude, phase, and mesor), the following cosinor mixed model was fitted independently for each herd:

$$Y_{\text{chjt}} = \beta_0 + \beta_{\cos}\, cos\left(\frac{2\pi t}{12}\right) + \beta_{\sin}\, sin\left(\frac{2\pi t}{12}\right) + u_{\text{cow(c)}} + v_{\text{year(j)}} + \varepsilon_{\text{chjt}},$$

where Y_{chjt} is milk composition variable for cow *c*, herd *h*, year *j*, and month *t*;

β_0 is overall intercept (mesor);

$\beta_{\cos}$ and $\beta_{\sin}$ are fixed coefficients for cosine and sine terms;

$u_{\text{cow(c)}} \sim N(0, \sigma^2_{\text{cow}})$ is the random intercept for cow c to account for repeated measures within animals;

$v_{\text{year(j)}} \sim N\left(0, \sigma^2_{\text{year}}\right)$ is the random intercept for year *j* to account for interannual variation, and

$\varepsilon_{\text{chjt}} \sim N(0, \sigma^2)$ is the residual error.

Temporal autocorrelation was assessed on standardized residuals using the ACF. When the absolute ACF at lags 1–3 exceeded 0.2, we applied an AR(1) residual correlation structure; otherwise, an independent residual structure was used.

The fit of the annual rhythm was assessed for each herd by a zero-amplitude F-test, comparing the full model (including the cosine and sine terms) with a reduced model

containing only the intercept and random effects. Herds with $P < 0.05$ were considered to exhibit a significant annual rhythm.

The mesor, amplitude, and acrophase were derived from the fixed-effect estimates according to Bourdon et al. (1995). Circular statistics were used to summarize the acrophase distribution across herds, with the circular mean and resultant vector length describing the degree of synchronization among herds.

To validate the consistency of the seasonal pattern detected in the national bulk-tank dataset, an additional cosinor mixed model was fitted using monthly herd averages combined across states. The model included fixed effects of state and the 12-mo cosinor terms (cosine and sine), as well as interaction terms between state and the seasonal components, allowing comparison of rhythm parameters among states. Random intercepts for herd and year were included to account for repeated measures and interannual variation:

$$Y_{\text{hijt}} = \beta_0 + \beta_{\text{state(i)}} + \beta_{\cos} \cos\left(\frac{2\pi t}{12}\right) + \beta_{\sin} \sin\left(\frac{2\pi t}{12}\right) + \beta_{\text{state(i):cos}} \cos\left(\frac{2\pi t}{12}\right) +$$

$$\beta_{\text{state(i):sin}} \sin\left(\frac{2\pi t}{12}\right) + u_{\text{herd(h)}} + v_{\text{year(j)}} + \varepsilon_{hijt},$$

where Y_{hijt} is milk composition variable for herd *h*, state *i*, year *j*, and month *t*;

β_0 is overall intercept (mesor);

$\beta_{\text{state(i)}}$ is fixed effect of state *i*;

$\beta_{\cos}$ and $\beta_{\sin}$ are fixed coefficients for cosine and sine terms;

$\beta_{\text{state(i):cos}}$ and $\beta_{\text{state(i):sin}}$ are interaction terms between state and seasonal components;

$u_{\text{herd(h)}} \sim N\left(0, \sigma^2_{\text{herd}}\right)$ is the random intercept for herd *h*;

$v_{\text{year(j)}} \sim N\left(0, \sigma^2_{\text{year}}\right)$ is the random intercept for year *j*; and

$\varepsilon_{\text{hijt}} \sim N(0, \sigma^2)$ is the residual error term.

Residual temporal autocorrelation was screened via ACF as above; models were re-fitted with an AR(1) residual structure when |ACF| at lags 1–3 exceeded 0.2, and otherwise retained independent residuals. This model allowed direct comparison of the fixed-effect coefficients (mesor, amplitude, and acrophase) among states and served to validate whether the seasonal rhythms observed in bulk-tank milk were consistent with those detected at the herd level.

Cow-level analysis

To investigate individual variation in seasonal rhythms and identify the proportion of cows exhibiting significant annual patterns in milk composition, we fitted the following cosinor mixed model separately for each cow. This approach allowed us to estimate cow-specific rhythm parameters while accounting for herd-level effects:

$$Y_{\mathrm{cjt}} = \beta_0 + \beta_{\cos}\, cos\left(\frac{2\pi t}{12}\right) + \beta_{\sin}\, sin\left(\frac{2\pi t}{12}\right) + u_{\mathrm{year\,(j)}} + \varepsilon_{\mathrm{cjt}},$$

where Y_{cjt} is milk composition variable for year *j*, and month *t* for the specific cow c being analyzed;

β_0 is overall intercept (mesor);

$\beta_{\cos}$ and $\beta_{\sin}$ are fixed coefficients for cosine and sine terms;

$u_{\mathrm{year(j)}} \sim N\left(0, \sigma^2_{\mathrm{year}}\right)$ is the random intercept for year *j* to account for interannual variation, and

$\varepsilon_{\mathrm{cjt}} \sim N(0, \sigma^2)$ is the residual error.

For each cow, residual ACF was inspected; if |ACF| at lags 1–3 exceeded 0.2, an AR(1) residual structure was used, otherwise residuals were modeled as independent. To assess whether individual cows exhibited a significant annual rhythm, a zero-amplitude F-test was applied to each cow by comparing the full model (including cosine and sine terms) with a reduced model containing only the intercept. Cows with $P < 0.05$

were classified as showing significant seasonality. The proportion of rhythmic cows was calculated for each milk component and summarized across states and production clusters.

To further assess the generalizability of the national Cosinor model, a combined cosinor mixed model was fitted to the entire cow-level dataset across states. Fixed effects of state and seasonal components (cosine and sine) were included, together with their interactions, to evaluate whether amplitude and phase of the annual rhythm differed among states. Random intercepts for herd, year, and cow were included to account for the hierarchical structure of the data:

$$Y_{\text{chijt}} = \beta_0 + \beta_{\text{state(i)}} + \beta_{\cos} cos\left(\frac{2\pi t}{12}\right) + \beta_{\sin} sin\left(\frac{2\pi t}{12}\right) + \beta_{\text{state(i):cos}} cos\left(\frac{2\pi t}{12}\right) +$$

$$\beta_{\text{state(i):sin}} sin\left(\frac{2\pi t}{12}\right) + u_{\text{cow(c)}} + v_{\text{herd(h)}} + w_{\text{year(j)}} + \varepsilon_{chijt},$$

where Y_{chijt} is milk composition variable for cow *c*, herd *h*, state *i*, year *j*, and month *t*;

β_0 is overall intercept (mesor);

$\beta_{\text{state(i)}}$ is fixed effect of state *i*;

$\beta_{\cos}$ and $\beta_{\sin}$ are fixed coefficients for cosine and sine terms;

$\beta_{\text{state(i):cos}}$ and $\beta_{\text{state(i):sin}}$ are interaction terms between state and seasonal components;

$u_{\text{cow(c)}} \sim N(0, \sigma^2_{\text{cow}})$ is the random intercept for cow *c*;

$v_{\text{herd(h)}} \sim N(0, \sigma^2_{\text{herd}})$ is the random intercept for herd *h*;

$w_{\text{year(j)}} \sim N(0, \sigma^2_{\text{year}})$ is the random intercept for year *j*; and

$\varepsilon_{\text{chijt}} \sim N(0, \sigma^2)$ is the residual error term.

emporal autocorrelation was evaluated on model residuals using ACF; models were re-estimated with an AR(1) residual structure when |ACF| at lags 1–3 exceeded 0.2, and retained independent residuals otherwise.. The presence and consistency of the annual rhythm across states were evaluated by zero-amplitude tests and by comparing the

rhythm-adjusted means and phases estimated from the cow-level model with those derived from the national dataset.

Results

Brazil Milk Composition Market Data

A significant annual rhythm ($P < 0.001$) was detected for both milk fat and protein content across all major dairy-producing states in Brazil (Table 1; Figures 1 and 2). The cosinor mixed model identified consistent seasonal oscillations with distinct state-level patterns in the rhythm-adjusted mean (mesor), amplitude, and acrophase. Zero-amplitude tests confirmed that the 12-month cosine model significantly described the seasonal variation in both traits in every state ($P < 0.001$).

Model comparison demonstrated a marked improvement in fit when seasonal terms were included. For milk fat, the residual sum of squares (RSS) decreased from 2.46 in the reduced model (state + year only) to 0.238 in the full cosinor model, representing an ≈90% reduction in unexplained variance. Similarly, for milk protein, RSS decreased from 0.763 to 0.148 (≈81% reduction), and for somatic cell count, RSS decreased from an average of 122,070 across states to 80,667 (≈34% reduction). The inclusion of cosinor terms consistently provided superior model fit compared with models containing only state and year effects.

Milk fat concentration

Fat concentration in all states fit a 12-mo rhythm ($P < 0.001$). The rhythm-adjusted mean (mesor) for fat differed among all states ($P < 0.05$), ranging from 3.60% in Goiás to 3.97% in Santa Catarina (Table 1). A clear latitudinal gradient was observed, with

304 southern states (e.g., Santa Catarina, Rio Grande do Sul) exhibiting higher average fat
305 concentrations than central-western states (e.g., Goiás, Minas Gerais).

306 The amplitude of the fat rhythm varied across states, ranging from 0.152
307 percentage units in Rio Grande do Sul to 0.192 percentage units in São Paulo. São Paulo
308 and Goiás exhibited the greatest seasonal variation (0.192 and 0.184 percentage units,
309 respectively) and did not differ from each other ($P > 0.10$). These amplitudes were greater
310 ($P < 0.05$) than those observed in Paraná and Rio Grande do Sul (0.157 and 0.152
311 percentage units, respectively), which formed a distinct low-amplitude group. Minas
312 Gerais and Santa Catarina displayed intermediate amplitudes (0.167 and 0.163 percentage
313 units, respectively) that were different from both the high-amplitude and low-amplitude
314 groups ($P < 0.05$).

315 The acrophase of the fat concentration rhythm revealed a distinct north-to-south
316 progression. The earliest peak occurred in Goiás (April 26), which was significantly
317 earlier ($P < 0.05$) than all other states. Minas Gerais peaked on May 10, intermediate
318 between Goiás and a cluster formed by São Paulo, Paraná, Santa Catarina, and Rio Grande
319 do Sul, which displayed similar acrophases between May 9 and May 12 ($P > 0.10$).

320 .

321 *Milk protein concentration*

322 Protein concentration also exhibited a significant 12-mo rhythm in all states ($P <$
323 0.001). The mesor for protein content differed among states ($P < 0.05$), with Goiás having
324 the highest mean (3.274%) and Paraná the lowest (3.239%; Table 1). Differences in mesor
325 were modest in magnitude and did not follow as clear a latitudinal gradient as observed
326 for fat, although central-western states (Goiás, Minas Gerais) tended to have slightly
327 higher mesors than São Paulo and Paraná, with Rio Grande do Sul and Santa Catarina
328 assuming intermediate positions.

The amplitude of the protein rhythm ranged from 0.088 to 0.102 percentage units. The amplitude was greatest in Rio Grande do Sul (0.102), which was similar ($P > 0.05$) to the amplitudes in Goiás (0.097), Minas Gerais (0.094), and São Paulo (0.095). This group of states with higher amplitudes differed ($P < 0.05$) from Paraná and Santa Catarina, which formed a homogeneous low-amplitude group (0.088 percentage units for both; $P > 0.10$).

A clear geographic progression was evident in the timing of the protein peak. The acrophase occurred earliest in Goiás (March 25). Minas Gerais and São Paulo formed an intermediate cluster, both peaking on April 8, later than Goiás but earlier than the southern states ($P < 0.05$). The southern states exhibited a progressive delay: Paraná peaked on May 7, Santa Catarina on May 20, and Rio Grande do Sul on May 30. The acrophases of these three southern states were all different from each other and from the central and southeastern states ($P < 0.05$), revealing a southward lag of approximately 60 days from the earliest to the latest peak.

.

Somatic cell count

Somatic cell count (SCC) demonstrated significant seasonal periodicity in all states ($P < 0.001$), with the cosinor model explaining substantially more variation than the reduced model across all regions. The mesor for SCC varied markedly among states ($P < 0.05$), ranging from 369.6×10^3 cells/mL in Goiás to 553.4×10^3 cells/mL in Rio Grande do Sul (Table 1). In general, southern states (Rio Grande do Sul, Santa Catarina) exhibited higher baseline SCC levels than central and southeastern states, whereas São Paulo (394.5×10^3 cells/mL) and Goiás had the lowest mesors.

The amplitude of SCC oscillation also showed substantial regional variation, ranging from 16.4×10^3 cells/mL in São Paulo to 33.0×10^3 cells/mL in Rio Grande do

Sul and Santa Catarina. Rio Grande do Sul and Santa Catarina formed a high-amplitude group (both 33.0 × 10^3 cells/mL; $P > 0.10$), which was significantly greater ($P < 0.05$) than the amplitudes observed in Minas Gerais (24.1 × 10^3 cells/mL), Paraná (22.3 × 10^3 cells/mL), and Goiás (18.6 × 10^3 cells/mL). São Paulo exhibited the lowest amplitude (16.4 × 10^3 cells/mL), significantly smaller than in all other states ($P < 0.05$).

The acrophase for SCC displayed a distinct seasonal pattern, with all states peaking during the summer months. Goiás peaked earliest (January 5), followed by Minas Gerais and São Paulo (January 13 and 14, respectively), which did not differ from each other ($P > 0.10$). The southern states peaked later, with Rio Grande do Sul reaching maximum SCC on February 10, Paraná on February 19, and Santa Catarina on February 23. This progression indicates an ≈50-day lag in SCC peak from the northernmost to southernmost states.

Seasonal and economic predictors of milk composition with state as a fixed effect

The comparison of 15 regression models revealed distinct patterns in the relative contribution of seasonal and economic factors to milk composition variation (Tables 2 and 3). For milk protein content, models incorporating cosinor terms consistently outperformed economic-only models. Among national protein models, the best-performing specification (Model 12: state + cos12 + sin12 + soybean price) explained 65.4% of the variance ($R^2 = 0.654$; Table 2). The cosinor-only model (Model 01: state + cos12 + sin12) already accounted for 65.3% of protein variation, indicating that seasonal patterns, captured by the 12-mo rhythm, represent the predominant source of variability.

Economic-only models for protein content (state plus price variables, without seasonal terms) showed much lower predictive power, with marginal R^2 values ranging from 0.026 to 0.237. Adding economic variables to the cosinor structure provided only

negligible improvements in model fit ($\Delta R^2 \leq 0.01$ compared with the cosinor-only baseline), and did not materially reduce RMSE.

For milk fat content, all cosinor-based national models exhibited exceptionally high explanatory power (marginal $R^2 \approx 0.935$), with minimal differences among them (Table 3). The cosinor-only specification with state and seasonal terms (Model 01) performed as well as or better than more complex models including combinations of soybean price, corn price, and state- or national-level milk prices. Economic-only models explained a substantially smaller fraction of the variation in fat content, with marginal R^2 values between 0.520 and 0.573 and RMSE values more than twice those of cosinor-based models.

Regional variability in seasonal and economic predictors of model performance

Analysis of model performance by state revealed substantial regional heterogeneity in the relative contribution of seasonal and economic drivers (Tables 4 and 5).

For milk protein content, within-state cosinor-only models already showed high predictive ability, with marginal R^2 ranging from 0.701 in Rio Grande do Sul to 0.850 in Minas Gerais (Table 4). Minas Gerais exhibited the highest predictability ($R^2 = 0.861$) when soybean and corn prices were added to the cosinor structure (Model 14), but the gain over the cosinor-only baseline was small ($\Delta R^2 = +0.011$). In Goiás, no economic-augmented model clearly outperformed the cosinor-only specification ($R^2 = 0.795$), indicating that seasonal patterns alone captured most of the explainable variation.

In contrast, the inclusion of economic variables yielded more noticeable, although still moderate, improvements in some southern states. In Rio Grande do Sul, marginal R^2 increased from 0.701 in the cosinor-only model to 0.762 when state-level milk price was

added (Model 10; $\Delta R^2 = +0.061$). Similarly, in Santa Catarina, adding the national milk price (Model 11) increased R^2 from 0.770 to 0.824 ($\Delta R^2 = +0.054$). Paraná and São Paulo showed intermediate behavior, with modest improvements when soybean and/or corn prices and milk price were included. Overall, however, seasonal cosinor terms remained the primary contributors to model performance, with economic predictors providing only incremental gains.

For milk fat content, all states exhibited excellent predictability (Table 5). Within-state cosinor-only models achieved marginal R^2 between 0.792 (Rio Grande do Sul) and 0.929 (Minas Gerais). The addition of economic variables slightly increased R^2 to a range of 0.820–0.933, with the largest improvements observed in Rio Grande do Sul (0.792 to 0.820; $\Delta R^2 = +0.028$), Santa Catarina (0.844 to 0.865; $\Delta R^2 = +0.021$), and São Paulo (0.896 to 0.918; $\Delta R^2 = +0.022$). In Minas Gerais and Paraná, the gains were minimal ($\Delta R^2 \leq +0.015$).

Herd-level cosinor analysis of milk composition and yield in Girolando herds

Herd-level mixed-effects cosinor models identified annual rhythms in milk fat, protein, somatic cell count (SCC), and milk yield in a subset of Girolando herds (Table 6). Among the 41 herds evaluated (mean 18 cows/herd), protein content showed the highest prevalence of seasonality, with 27 herds (65.9%) classified as rhythmic ($P < 0.05$), followed by milk yield (14 herds; 34.1%), fat (13 herds; 31.7%), and SCC (9 herds; 22.0%).

The magnitude and variability of the seasonal oscillations differed among traits. For fat, the mean herd-level amplitude was 0.147 percentage units and the coefficient of variation (CV) of the amplitude was 59.8% (Table 6). Protein had a mean amplitude of 0.116 percentage units and an amplitude CV of 54.4%. Somatic cell count showed a mean

amplitude of 253.7 × 10^3 cells/mL and the highest amplitude CV (86.9%). For milk yield, the mean amplitude of the annual rhythm was 2.05 kg/d and the amplitude CV was 71.5%.

The circular mean acrophase for fat occurred in mid-June (June 14), whereas protein peaked at the end of May (May 30; Table 6). Milk yield reached its mean acrophase on August 4, and SCC peaked on October 16. Circular concentration values were 0.65 for protein, 0.41 for fat, 0.29 for milk yield, and 0.09 for SCC, indicating tighter clustering of acrophases for protein and more dispersed acrophases for SCC.

Conditional R^2 values for the herd-level cosinor models were 0.273 for fat, 0.380 for protein, 0.344 for SCC, and 0.337 for milk yield (Table 6), indicating that the combination of fixed cosinor terms and the random effects included in the herd-level models explained 27.3 to 38.0% of the total variance in these traits.

Herd-level differences among states

When herd-level cosinor parameters were summarized by state, clear regional patterns were observed among Minas Gerais, Paraná, and São Paulo (Table 7). For milk fat, the proportion of herds exhibiting significant annual rhythms was similar across states, ranging from 30.0% in Minas Gerais and São Paulo to 40.0% in Paraná. However, mean fat amplitude differed among states, being 0.136 percentage units in Minas Gerais, 0.127 in Paraná, and 0.185 in São Paulo. The mean acrophase for fat occurred in early June in Minas Gerais (June 5) and Paraná (June 6) and in early July in São Paulo (July 5). Conditional R^2 values for fat ranged from 0.240 in Minas Gerais to 0.366 in São Paulo.

For protein, seasonal rhythms were frequent in all 3 states, with 65.4% of herds classified as rhythmic in Minas Gerais, 60.0% in Paraná, and 70.0% in São Paulo. Mean protein amplitude was 0.115 percentage units in Minas Gerais, 0.087 in Paraná, and 0.132 in São Paulo. Minas Gerais and Paraná shared the same mean acrophase date (May 26),

whereas São Paulo had a later mean acrophase (June 9). Conditional R^2 values for protein were similar among states and ranged from 0.360 to 0.385.

For SCC, the proportion of herds with significant annual rhythms was lower than for fat and protein, with 26.9% in Minas Gerais, 20.0% in Paraná, and 10.0% in São Paulo. Among rhythmic herds, mean SCC amplitude was 242.496 × 10^3 cells/mL in Minas Gerais, 160.533 × 10^3 cells/mL in Paraná, and 329.352 × 10^3 cells/mL in São Paulo (Table 7). The mean acrophase for SCC occurred on September 27 in Minas Gerais, October 1 in Paraná, and January 1 in São Paulo. Conditional R^2 values ranged from 0.326 to 0.389 across states.

For milk yield, the proportion of rhythmic herds was 30.8% in Minas Gerais, 20.0% in Paraná, and 50.0% in São Paulo. Mean amplitudes of the annual yield rhythm were 1.923 kg/d in Minas Gerais, 1.481 kg/d in Paraná, and 2.674 kg/d in São Paulo. The mean acrophase dates for milk yield were August 9 in Minas Gerais, October 8 in Paraná, and November 9 in São Paulo. Conditional R^2 values for milk yield varied from 0.267 in Paraná to 0.358 in São Paulo.

Validation of herd-level rhythms with a combined herd–state cosinor model

To evaluate herd-level rhythms in a joint framework and compare parameters across states, a combined herd–state cosinor mixed-effects model was fitted for Minas Gerais, Paraná, and São Paulo (Table 8). In this validation model, mesor, amplitude, and acrophase were estimated at the herd–state level, and a single global zero-amplitude test and conditional R^2 were obtained for each trait.

For milk fat, the global zero-amplitude test indicated a significant annual rhythm at the herd–state level ($P = 0.013$), with a conditional R^2 of 0.186. The herd–state mesor for fat was 3.7151% in Minas Gerais, 3.8144% in Paraná, and 3.8066% in São Paulo.

Corresponding amplitudes were 0.0638 percentage units in Minas Gerais, 0.0287 in Paraná, and 0.0979 in São Paulo. The estimated acrophase for fat occurred on June 9 in Minas Gerais, June 27 in Paraná, and July 4 in São Paulo.

For protein, the combined herd–state model also detected a significant annual rhythm ($P < 0.001$), with conditional $R^2 = 0.228$. Protein mesors were 3.3678% in Minas Gerais, 3.3327% in Paraná, and 3.2977% in São Paulo. Amplitudes were 0.0436 percentage units in Minas Gerais, 0.0587 in Paraná, and 0.0612 in São Paulo. The corresponding acrophase dates were May 10 for Minas Gerais, May 24 for Paraná, and June 10 for São Paulo.

For SCC, the validation model yielded $P = 0.061$ for the global zero-amplitude test and a conditional R^2 of 0.274. Herd–state SCC mesors were 620.90×10^3 cells/mL in Minas Gerais, 757.91×10^3 cells/mL in Paraná, and $1{,}062.99 \times 10^3$ cells/mL in São Paulo. Amplitudes were 130.05×10^3 cells/mL in Minas Gerais, 74.17×10^3 cells/mL in Paraná, and 173.07×10^3 cells/mL in São Paulo. The estimated acrophase dates for SCC were July 12 in Minas Gerais, September 22 in Paraná, and October 15 in São Paulo.

These combined-model results summarize herd-level mesor, amplitude, and acrophase for each state, while P-values and conditional R^2 are common within trait because they derive from a single joint mixed-effects model fitted across states.

Cow-level cosinor analysis of milk composition and yield in Girolando cows

Cow-level cosinor models identified a substantial proportion of Girolando cows with detectable annual rhythms in milk fat, protein, somatic cell count (SCC), and milk yield (Table 9). Across 41 herds (mean 18 cows/herd; mean 11.2 mo of records per cow), 147 cows (20.5%) showed a significant annual rhythm in fat content, 331 cows (46.2%) in protein, 159 cows (22.2%) in SCC, and 370 cows (51.6%) in milk yield ($P < 0.05$ for

the zero-amplitude F-test). Among cows classified as rhythmic, the mean amplitude of the fitted 12-mo cosine function was 0.350 percentage units for fat and 0.215 percentage units for protein, with amplitude coefficients of variation (CV) of 59.6% and 53.2%, respectively. For SCC, the mean amplitude was 462.037 × 10^3 cells/mL with an amplitude CV of 120.9%, whereas for milk yield the mean amplitude was 5.472 kg/d with an amplitude CV of 57.2% (Table 9).

The circular mean acrophase for fat occurred on June 19 and for protein on June 21, whereas SCC peaked on October 15 and milk yield on July 8. Circular concentration values, summarizing the dispersion of acrophases, were 0.21 for fat, 0.24 for protein, 0.07 for SCC, and 0.08 for milk yield, indicating that acrophases were more tightly clustered for protein and fat than for SCC and milk yield. Mean adjusted R^2 values for individual cow-level cosinor models were 0.213 for fat, 0.309 for protein, 0.216 for SCC, and 0.321 for milk yield, showing that the 12-mo cosine function accounted for a moderate proportion of within-cow temporal variation among cows with significant rhythms (Table 9).

State-level differences in cow-level rhythmicity

When cow-level cosinor parameters were summarized by state, clear differences were observed among Minas Gerais, Paraná, and São Paulo (Table 10). For fat, the proportion of cows with significant annual rhythms ranged from 19.8% in Minas Gerais (94 of 475 cows) to 22.5% in Paraná (23 of 102 cows) and 21.6% in São Paulo (30 of 139 cows). Among rhythmic cows, mean fat amplitude was highest in Minas Gerais (0.599 percentage units), intermediate in São Paulo (0.576), and lowest in Paraná (0.456). The mean acrophase for fat occurred on July 9 in Minas Gerais, August 12 in Paraná, and July

1 in São Paulo. Mean adjusted R^2 values for fat models ranged from 0.192 in Paraná to 0.223 in São Paulo, with Minas Gerais at 0.214.

For protein, the prevalence of rhythmic cows was higher than for fat in all states. In Minas Gerais, 226 of 476 cows (47.5%) were rhythmic, compared with 46 of 102 cows (45.1%) in Paraná and 59 of 139 cows (42.4%) in São Paulo. Mean amplitudes were 0.293, 0.265, and 0.287 percentage units in Minas Gerais, Paraná, and São Paulo, respectively, and mean acrophases occurred on June 12, July 19, and June 9. Mean adjusted R^2 values for protein were 0.325 in Minas Gerais, 0.260 in Paraná, and 0.326 in São Paulo (Table 10).

For SCC, 109 cows (22.9%) in Minas Gerais, 22 cows (21.6%) in Paraná, and 28 cows (20.1%) in São Paulo showed significant annual rhythms. Mean SCC amplitudes among rhythmic cows were 677.449 × 10^3 cells/mL in Minas Gerais, 512.876 × 10^3 cells/mL in Paraná, and 718.172 × 10^3 cells/mL in São Paulo. Mean acrophases for SCC occurred on October 28 in Minas Gerais, October 15 in Paraná, and September 22 in São Paulo, and mean adjusted R^2 values ranged from 0.208 to 0.220 across states.

For milk yield, rhythmic cows represented 49.6% of the cows in Minas Gerais (236 of 476 cows), 52.0% in Paraná (53 of 102 cows), and 58.3% in São Paulo (81 of 139 cows). Mean amplitudes of the annual yield rhythm were 9.471 kg/d in Minas Gerais, 7.120 kg/d in Paraná, and 6.560 kg/d in São Paulo. Mean acrophases occurred on July 27, July 25, and July 22 in Minas Gerais, Paraná, and São Paulo, respectively. The mean adjusted R^2 for milk-yield cosinor models was 0.328 in Minas Gerais, 0.272 in Paraná, and 0.322 in São Paulo, indicating broadly similar goodness-of-fit across states (Table 10).

Cow-level mixed-effects validation model across states

A combined cow-level mixed-effects cosinor validation model, fitted jointly across Minas Gerais, Paraná, and São Paulo with random intercepts for cow, herd, and year, was used to obtain state-specific mesor, amplitude, and acrophase estimates for each trait (Table 11). For fat, the global zero-amplitude test provided strong evidence of annual seasonality at the cow level ($P < 0.001$), with mesors of 3.7257, 3.8233, and 3.8003 percentage units in Minas Gerais, Paraná, and São Paulo, respectively. Corresponding amplitudes were 0.0860, 0.0579, and 0.1212 percentage units, and acrophases occurred on June 6, June 28, and June 26. The conditional R^2 of the combined cow-level model for fat was 0.248.

For protein, the validation model also supported a pronounced annual rhythm ($P < 0.001$), with mesors of 3.3749% (Minas Gerais), 3.3764% (Paraná), and 3.3047% (São Paulo), and amplitudes of 0.0565, 0.0681, and 0.0815 percentage units, respectively (Table 11). The cow-level acrophase for protein occurred on May 19 in Minas Gerais, June 13 in Paraná, and June 26 in São Paulo, and the conditional R^2 for the protein model was 0.326.

For SCC, the global zero-amplitude test from the combined cow-level model did not support a significant annual rhythm ($P = 0.903$). Nonetheless, the fitted model yielded mesors of 443.81 × 10^3 cells/mL in Minas Gerais, 758.34 × 10^3 cells/mL in Paraná, and 933.26 × 10^3 cells/mL in São Paulo, with corresponding amplitudes of 27.35, 9.67, and 18.36 × 10^3 cells/mL and acrophases on October 29, January 15, and October 1, respectively. The conditional R^2 of the SCC validation model was 0.389 (Table 11).

Discussion

This study demonstrated, across multiple organizational levels, the presence of well-defined annual rhythms in milk composition and somatic cell count (SCC) of dairy cows in Brazil. At the market level, national bulk-tank data revealed robust seasonal variation in fat, protein, and SCC in all major dairy states, with substantial reductions in residual variance when cosinor terms were included in the models, consistent with circannual rhythms in milk fat and protein described in North American herds (Salfer et al., 2019, 2020) with marked seasonal patterns in milk yield, composition, and SCC in intensive Holstein herds in China (Yang et al., 2013), and with earlier reports of seasonal shifts in milk composition in both temperate and subtropical systems (Noro et al., 2006; Niu et al., 2014). The influence of season, particularly heat stress, as a key driver of these rhythms is well documented: in the intensive Chinese herd studied by Yang et al. (2013), daily milk yield, fat, protein, and total solids reached their lowest values in early summer even though cows received a total mixed ration based on the same feedstuffs throughout the year, indicating that environmental load rather than major changes in diet formulation explained much of the seasonal variation.

In parallel, herd- and cow-level analyses in Girolando herds confirmed that these annual rhythms are not only an aggregate market phenomenon but also manifest within herds and individual cows, albeit with greater heterogeneity among production units and animals, consistent with the significant interaction between seasonal change and parity on milk yield, milk lactose, somatic cell traits, and milk production loss reported by Yang et al. (2013). This aligns with multi-level analyses reported for Holstein cows in the United States (Salfer et al., 2019, 2020) and with experimental evidence for photoperiod-mediated regulation of lactation physiology (Dahl et al., 2000, 2012).

A central feature of this work is the combined use of 2 large and complementary data sources: (1) the official bulk-tank milk composition database from the Brazilian Ministry of Agriculture and Livestock, derived from routine payment-for-quality testing, and (2) the performance records from the Brazilian Girolando Breeders Association, including test-day milk recording and productivity data from herds enrolled in genetic improvement programs. Girolando is the predominant dairy genetic group in Brazilian tropical and subtropical regions, and animals of this breed are estimated to account for approximately 80% of the country's milk production (Canaza-Cayo et al., 2015). The breed was developed in Brazil from systematic crosses between Holstein and Gir cattle to combine high milk yield with adaptation to tropical conditions (Canaza-Cayo et al., 2016).

Thus, although the Girolando herds analyzed here represent relatively well-organized, performance-recorded herds with higher-than-average levels of technification, the seasonal patterns described at herd and cow levels are likely broadly representative of a large and economically important segment of Brazilian dairy production under tropical and subtropical conditions. At the same time, the fact that the Girolando database is restricted to herds enrolled in breed association programs is both a strength, because of the standardized, quality-controlled data, and a limitation, as it excludes herds with lower recording intensity, other specialized breeds, and non-standard crossbreeding schemes.

The mechanisms underlying these seasonal patterns most likely reflect a complex interaction between environmental drivers, physiological responses, and management practices (Bernabucci et al., 2010; Becker et al., 2020). The latitudinal gradients in acrophase for fat and protein follow Brazilian climatic patterns, and similar geographic differences in the timing and amplitude of annual rhythms of milk fat and protein have been described across US milk markets and herds (Salfer et al., 2019, 2020), suggesting

that thermoregulation and changes in energy metabolism in response to seasonal heat load are key determinants of the observed rhythms (Bernabucci et al., 2010; Wheelock et al., 2010). Periods of high heat stress are known to reduce feed intake and shift nutrients away from production toward maintenance of homeothermy, thereby depressing milk yield and altering component synthesis (West, 2003; Collier et al., 2006; Bernabucci et al., 2010; Becker et al., 2020).

In parallel, seasonal fluctuations in the quantity and nutritive value of forages, particularly in pasture-based and semi-confinement systems that still account for much of Brazilian milk production, modify rumen fermentation patterns and the profile of nutrients supplied to the mammary gland (Noro et al., 2006; Marcondes et al., 2014; Nogara et al., 2022). Studies using bulk-tank and farm-level data in southern and southeastern Brazil have consistently reported higher milk fat and protein concentrations and more favorable SCC and bacterial counts during the colder months, when the combination of improved thermal comfort and better forage quality, including the use of temperate grasses, is expected to support greater nutrient intake and efficiency (Noro et al., 2006; Marcondes et al., 2014; Nogara et al., 2022). The combination of direct heat stress and these indirect nutritional effects of seasonally variable pastures and conserved forages likely contributes to the consistency of the annual rhythms observed across market, herd, and cow levels (West, 2003; Bernabucci et al., 2010; Becker et al., 2020). Experimental and observational studies in ruminants have shown that both heat stress and changes in diet composition can differentially affect milk fat and protein synthesis through alterations in rumen function, endocrine status, and peripheral tissue metabolism (Bernabucci et al., 2010; Piccioli-Cappelli et al., 2014).

The bulk-tank analysis at the national level revealed clear geographic gradients in milk composition. Annual mesors for fat were systematically higher in southern states

649 than in Goiás and Minas Gerais, whereas amplitudes and acrophases highlighted not only
650 differences in the average level of the cycle but also its intensity and timing. Similar,
651 albeit slightly smaller, amplitudes were observed for protein, with a marked spatial
652 progression in peak timing, starting in Goiás in late March and shifting gradually toward
653 the southern states by late May. In contrast, SCC showed higher mesors in the South and
654 peaks concentrated in mid to late summer, with a lag of several weeks from the
655 northernmost to the southernmost states. This pattern is consistent with the well-
656 documented seasonality of mastitis and SCC in temperate and subtropical dairying, where
657 warmer, more humid periods are associated with increased environmental pathogen
658 pressure and higher SCC (Olde Riekerink et al., 2007; Ferreira and De Vries, 2015).

659 Together, these results suggest that, beyond large-scale climatic and photoperiodic
660 forcing, regional production systems (e.g., pasture vs. partial or total confinement), forage
661 conservation strategies, reproductive calendars, and udder health management contribute
662 to shaping the observed state-specific rhythms. Consistent with this interpretation, the
663 comparatively low conditional R^2 values obtained for SCC at herd and cow levels, relative
664 to fat and protein, indicate that annual seasonality explains only a modest fraction of its
665 temporal variance, leaving substantial room for episodic environmental challenges,
666 mastitis outbreaks, and management-related factors to drive within-year fluctuations in
667 udder health.

668 Our findings are consistent with recent cosinor-based analyses from the Northern
669 Hemisphere that have highlighted the existence of endogenous circannual patterns in milk
670 composition. In US Holstein herds, Salfer et al. (2019, 2020) used rhytmometric models
671 to show that milk fat and protein follow stable annual rhythms, with peaks in December–
672 January closely aligned with the winter solstice, largely independent of short-term
673 nutritional changes and episodic heat stress.

In those studies, bulk-tank fat and protein amplitudes typically ranged from about 0.07 to 0.14 and 0.08 to 0.12 percentage units, respectively (Salfer 2019, 2020). In our Brazilian bulk-tank data, peak fat and protein also occurred near the winter solstice of the corresponding hemisphere, with maximum values between April and June, and amplitudes of approximately 0.16 to 0.20 for fat and 0.09 to 0.11 for protein. Thus, the timing of the annual cycles appears to be conserved across hemispheres, consistent with a photoperiod-driven component of the rhythm (Dahl et al., 2000; Lincoln et al., 2006; Salfer et al., 2019). However, the slightly larger fat amplitudes in Brazil suggest a stronger contribution of seasonal variation in forage quality and feeding strategies, particularly in grazing-dominant regions (Palmquist et al., 1993). Interestingly, as in Florida in the work of Salfer et al. (2020), our results showed larger fat and protein amplitudes in states closer to the equator (Goiás, Minas Gerais, São Paulo) and smaller amplitudes in the higher-latitude southern states. This pattern does not fit a simple day-length gradient and reinforces the idea that regional production systems, heat load, and nutritional seasonality can modulate or override the purely photoperiodic component, especially in tropical and subtropical climates.

The regression analyses incorporating seasonal and economic predictors with fixed effects of state further emphasized the dominance of annual rhythms over short-term economic drivers. At the national level, cosinor terms with a 12-mo period captured the bulk of the temporal variability in fat and protein, whereas the inclusion of soybean, corn, and milk prices, either at national or state level, yielded only modest incremental gains in R^2 and negligible reductions in RMSE. Similar patterns emerged in within-state models: in nearly all cases, specifications that included only state (or herd), cos12, and sin12 already exhibited high predictive power, and adding feed or milk price variables produced only small refinements. These findings are consistent with economic and time-

699 series analyses showing that farmgate milk prices display their own seasonal component
700 driven largely by seasonal fluctuations in milk supply and in fat and protein content
701 (Olipra, 2019), and that world milk and feed prices share common cyclical dynamics and
702 long-run co-integration (Hansen and Li, 2017). Together, this evidence supports the
703 interpretation that a substantial fraction of the temporal variability in milk and feed prices
704 reflects the same underlying climatic, nutritional, and supply-demand forces that generate
705 the annual rhythms in milk composition, which helps to explain why, once the 12-mo
706 cosinor terms are included, economic variables contribute relatively little additional
707 temporal structure to the models (Sicheski et al., 2020).

708 Beyond their statistical interest, the predictable seasonal rhythms documented
709 here have practical implications for the dairy industry. On the processing side, knowledge
710 of the timing and magnitude of peaks in solids content can be used to plan plant capacity,
711 product mix, and marketing strategies over the year, because seasonal shifts in milk
712 composition and heat stability directly affect processing performance and product quality
713 (Li et al., 2019; Hayes et al., 2023). Periods with higher expected fat and protein could
714 be prioritized for manufacturing cheeses and other high-solids products, given the strong
715 influence of milk solids concentration and the protein-to-fat ratio on cheese yield and
716 composition (Guinee et al., 2007). At the farm level, recognizing these rhythms can guide
717 nutritional and reproductive management, such as strategic supplementation leading into
718 periods when component yields are naturally lower, or aligning calving patterns to better
719 exploit months with more favorable climatic and nutritional conditions (Salfer et al.,
720 2019; Hayes et al., 2023). For policy-makers and milk buyers, acknowledging the
721 inherent seasonality of composition can support more equitable payment schemes and
722 contract structures that account for natural within-year variation, minimizing undue
723 penalties when intrinsic milk quality is seasonally depressed and building on evidence

724 that quality-based premium programs and component pricing systems can substantially
725 modify observed milk quality and herd profitability (Bailey et al., 2005; Nightingale et
726 al., 2008).

727 At the herd level, mixed-effects cosinor models showed that a majority of
728 Girolando herds expressed detectable annual rhythms in protein and milk yield, whereas
729 seasonal patterns in fat and especially SCC were less prevalent. Average herd-level
730 amplitudes for protein and fat were modest in absolute terms (≈0.10–0.15 percentage
731 units) but exhibited high coefficients of variation, indicating substantial between-herd
732 heterogeneity in the strength of seasonal oscillations. Protein stood out not only for its
733 higher prevalence of rhythmic herds but also for its greater angular concentration of
734 acrophases and higher conditional R^2 values, suggesting that in many herds protein
735 seasonality is a structured and consistent component of within-year variation. In contrast,
736 SCC amplitudes were large in absolute magnitude but had high relative variability and
737 low phase concentration, reinforcing the weaker and poorly synchronized nature of SCC
738 seasonality across herds and aligning with evidence that mastitis and udder health traits
739 are driven by multifactorial and often episodic environmental and management-related
740 challenges rather than by smooth, herd-level seasonal cycles (Olde Riekerink et al., 2007;
741 Ferreira and De Vries, 2015).

742 The choice of a 12-mo cosinor model with fixed period aligns with long-standing
743 approaches in animal production science to describe seasonal and lactation curves
744 (Bourdon et al., 1995; Salfer et al., 2019, 2020). Classic work in temperate regions has
745 shown the usefulness of sinusoidal and related parametric models for capturing cyclic
746 patterns in milk yield and composition (Macciotta et al., 2005). However, in tropical and
747 subtropical environments, where rainfall seasonality may be more pronounced than
748 temperature seasonality and where forage growth dynamics are highly nonlinear, a strictly

sinusoidal form may not fully capture the complexity of production responses. More flexible frameworks, such as random-regression and reaction-norm models that incorporate environmental or climatic covariates directly (e.g., temperature–humidity index, region, and season of calving), have been successfully used in countries with similar climates to partition climatic, nutritional, and management effects on milk production and udder health (Bignardi et al., 2015; Hammami et al., 2015; Evangelista et al., 2024), pointing to a promising direction for future Brazilian studies.

State-level stratification at herd and cow levels showed that national patterns emerge from different combinations of rhythmic prevalence, amplitude, and phase in Minas Gerais, Paraná, and São Paulo. In general, São Paulo tended to exhibit larger seasonal amplitudes for fat, protein, SCC, and milk yield in rhythmic herds and cows, with somewhat later acrophases for fat, protein, and yield compared with Minas Gerais and, in some instances, Paraná. Minas Gerais, in turn, contributed the largest absolute number of herds and cows and tended to show slightly earlier peaks in protein and fat. These nuances are consistent with regional differences in calving distribution, the relative importance of winter and summer pastures, the intensity of conserved forage use, and the design of transition and summer-feeding programs within Girolando herds in the Southeast and South.

From a breeding perspective, the finding that a substantial proportion of cows exhibit significant annual rhythms, particularly for protein and milk yield, suggests the presence of exploitable individual variation in seasonal resilience. Similar to resilience indicators derived from longitudinal milk yield deviations that have been shown to be heritable and genetically associated with responses to environmental disturbances (Poppe et al., 2021; Chen et al., 2023), these seasonal patterns indicate that stability of yield and composition across seasons can be treated as a selection objective. Breeding schemes are

increasingly incorporating resilience and robustness traits alongside production in their breeding goals to cope with climate variability (Bengtsson et al., 2022; Hayes et al., 2023). Selection for "seasonal resilience", understood as the ability to maintain production and milk quality with reduced within-year fluctuation, may therefore be an attractive strategy to deal with ongoing climate change and increasing environmental variability (Silpa et al., 2021; Hayes et al., 2023). The long-term Girolando recording database, with repeated test-day records across many years and herds, is well suited for deriving such resilience indicators and for estimating genetic parameters and, potentially, genomic markers associated with tolerance to specific environmental challenges (Poppe et al., 2021; Chen et al., 2023).

The combined validation models at herd and cow levels, fitted jointly for the 3 states, provided a coherent statistical framework for summarizing regional differences while leveraging information across states. In these models, state-specific mesors, amplitudes, and acrophases were derived from fixed effects, whereas a single global zero-amplitude test and a single conditional R^2 per trait captured the overall evidence for seasonality and the total variance explained. The fact that P-values and conditional R^2 are identical across states within trait is not a methodological flaw but rather a direct consequence of the joint modeling strategy: the zero-amplitude test evaluates whether the cosinor coefficients differ from zero at the population level, and state-level differences manifest only through their respective mesor, amplitude, and phase estimates. This combined approach improves the precision of state-specific parameter estimates by pooling information, but at the cost of assuming a common variance–covariance structure across states, an assumption that should be considered when interpreting small between-state differences.

At the individual level, cow-specific cosinor models showed that approximately half of Girolando cows had detectable annual rhythms in protein and milk yield, whereas the prevalence of rhythmicity for fat and SCC was lower. Mean adjusted R^2 values around 0.21–0.32 indicate that annual seasonality explains an important, but not dominant, fraction of within-cow temporal variance, which is expected given the concurrent effects of lactation stage, pregnancy, health events, and management changes. The combined cow-level mixed model by state confirmed strong annual rhythms for fat and protein at the population level, whereas for SCC the global zero-amplitude test did not support a consistent annual oscillation, despite the presence of subgroups of cows with individually significant rhythms. This discrepancy highlights the heterogeneity of SCC patterns: some cows exhibit clear seasonal fluctuations, but the direction, timing, and magnitude are variable enough that the population-average signal is attenuated.

Despite the large sample sizes and the use of mixed models, several limitations should be acknowledged. First, both the bulk-tank and Girolando datasets are observational, with no experimental control over diet, housing, or reproductive management. Consequently, although annual rhythms are clearly characterized descriptively, causal attribution to specific drivers such as photoperiod, temperature–humidity index, forage availability, or feeding protocols is not possible. Second, the cosinor models assume a strictly sinusoidal form with a fixed 12-mo period, which does not allow for asymmetric rise and decline, multiple peaks, or interannual shifts in phase. Incorporating higher-order harmonics, nonlinear structures, or more flexible time-series approaches (e.g., generalized additive mixed models) could capture finer aspects of seasonal variation in future work. Third, the linear mixed models used for the economic analyses and for herd- and cow-level assessments assume linear relationships, approximate normality of residuals, and relatively simple temporal correlation structures

handled indirectly via random effects. Residual autocorrelation, multicollinearity among soybean, corn, and milk prices, and structural changes over the 11-yr period were not explicitly modeled. Biologically, the absence of explicit environmental covariates (e.g., temperature–humidity index, rainfall, photoperiod) and production-system descriptors (e.g., grazing vs. confinement, use of compost-barn or freestall, forage-to-concentrate ratio) limits our ability to separate endogenous rhythmic components from those directly induced by exogenous climatic and management factors.

Finally, the scope of the databases constrains the generalizability of the results. The bulk-tank data cover only 6 states, albeit ones that account for a large share of national milk production, and the Girolando records encompass 3 states with a long dairy tradition but exclude emerging production frontiers. Moreover, Girolando herds participating in milk recording and breeding programs tend to be more technically advanced, with more complete records and, on average, better productive and sanitary performance than the national mean. Thus, the seasonal patterns described here should be interpreted as representative of commercial, technified dairy systems under tropical and subtropical Brazilian conditions, rather than as a full portrait of all dairy systems and regions in the country.

Conclusions

This study provides robust evidence that milk fat and protein in Brazilian dairy systems follow a well-defined circannual rhythm that is conserved across organizational levels and largely independent of short-term economic fluctuations. At the national level, 12-mo cosinor terms captured most of the temporal variation in bulk-tank fat and protein and revealed coherent geographic gradients in mesor, amplitude, and acrophase among

major dairy states, whereas the inclusion of milk and feed price variables yielded only marginal improvements in model performance.

Herd- and cow-level cosinor analyses in Girolando herds confirmed that these annual rhythms are not merely an aggregate market phenomenon, but are also expressed within herds and individual cows, particularly for protein and milk yield, whereas SCC showed weaker, more heterogeneous, and less synchronized patterns. Together, these findings support the existence of a stable circannual organization of milk composition under tropical and subtropical Brazilian conditions, modulated primarily by climatic and production-system factors rather than by economic variability, and highlight exploitable between-herd and between-cow differences in seasonal responses.

Notes

The authors are grateful to the Brazilian Girolando Breeders Association (Associação Brasileira dos Criadores de Girolando, Uberaba, MG, Brazil) for providing access to the milk-recording database and for their collaboration in making these analyses possible.

References

Bailey, K.W., C.M. Jones, and A.J. Heinrichs. 2005. Economic returns to Holstein and Jersey herds under multiple component pricing. J Dairy Sci 88:2269–2280. https://doi.org/10.3168/jds.S0022-0302(05)72903-9.

Becker, C.A., R.J. Collier, and A.E. Stone. 2020. Invited review: Physiological and behavioral effects of heat stress in dairy cows. J Dairy Sci 103:6751–6770. https://doi.org/10.3168/jds.2019-17929.

Bengtsson, C., J.R. Thomasen, M. Kargo, A. Bouquet, and M. Slagboom. 2022. Emphasis on resilience in dairy cattle breeding: Possibilities and consequences. J Dairy Sci 105:7588–7599. https://doi.org/10.3168/jds.2021-21049.

Bernabucci, U., L. Basiricò, P. Morera, D. Dipasquale, A. Vitali, F. Piccioli Cappelli, and L. Calamari. 2015. Effect of summer season on milk protein fractions in Holstein cows. J Dairy Sci 98:1815–1827. https://doi.org/10.3168/jds.2014-8788.

Bernabucci, U., N. Lacetera, L.H. Baumgard, R.P. Rhoads, B. Ronchi, and A. Nardone. 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal 4:1167–1183. https://doi.org/10.1017/S175173111000090X.

Bignardi, A.B., L. El Faro, R.J. Pereira, D.R. Ayres, P.F. Machado, L.G. de Albuquerque, and M.L. Santana. 2015. Reaction norm model to describe environmental sensitivity across first lactation in dairy cattle under tropical conditions. Trop Anim Health Prod 47:1405–1410. https://doi.org/10.1007/s11250-015-0878-4.

Bourdon, L., A. Buguet, M. Cucherat, and M.W. Radomski. 1995. Use of a Spreadsheet Program for Circadian Analysis of Biological/Physiological Data . Aviat Space Environ Med 66:787–791.

Canaza-Cayo, A.W., J.A. Cobuci, P.S. Lopes, R. de Almeida Torres, M.F. Martins, D. dos Santos Daltro, and M.V.G. Barbosa da Silva. 2016. Genetic trend estimates for milk yield production and fertility traits of the Girolando cattle in Brazil. Livest Sci 190:113–122. https://doi.org/10.1016/j.livsci.2016.06.009.

Canaza-Cayo, A.W., P.S. Lopes, M.V.G.B. Da Silva, R. De Almeida Torres, M.F. Martins, W.A. Arbex, and J.A. Cobuci. 2015. Genetic parameters for milk yield and lactation persistency using random regression models in girolando cattle. Asian-Australas J Anim Sci 28:1407–1418. https://doi.org/10.5713/ajas.14.0620.

Chen, S.Y., J.P. Boerman, L.S. Gloria, V.B. Pedrosa, J. Doucette, and L.F. Brito. 2023. Genomic-based genetic parameters for resilience across lactations in North American Holstein cattle based on variability in daily milk yield records. J Dairy Sci 106:4133–4146. https://doi.org/10.3168/jds.2022-22754.

Chew, B.P., P. V. Malven, R.E. Erb, C.N. Zamet, M.F. D'Amico, and V.F. Colenbrander. 1979. Variables Associated with Peripartum Traits in Dairy Cows. IV. Seasonal Relationships among Temperature, Photoperiod, and Blood Plasma Prolactin. J Dairy Sci 62:1394–1398. https://doi.org/10.3168/jds.S0022-0302(79)83435-9.

Collier, R.J., G.E. Dahl, and M.J. Vanbaale. 2006. Major advances associated with environmental effects on dairy cattle. J Dairy Sci 89:1244–1253. https://doi.org/10.3168/jds.S0022-0302(06)72193-2.

Dahl, G.E., B.A. Buchanan, and H.A. Tucker. 2000. Photoperiodic effects on dairy cattle: A review. J Dairy Sci 83:885–893. https://doi.org/10.3168/jds.S0022-0302(00)74952-6.

Dahl, G.E., S. Tao, and I.M. Thompson. 2012. Lactation biology symposium: Effects of photoperiod on mammary gland development and lactation. J Anim Sci 90:755–760. https://doi.org/10.2527/jas.2011-4630.

Evangelista, A.F., R. Martins, A.A. Valotto, L.T. Dias, and R. de Almeida Teixeira. 2024. Environmental factors on the prediction of the lactation curve of Holstein cows. Pesqui Agropecu Bras 59. https://doi.org/10.1590/S1678-3921.PAB2024.V59.03366.

Ferreira, F.C., and A. De Vries. 2015. Effects of season and herd milk volume on somatic cell counts of Florida dairy farms. J Dairy Sci 98:4182–4197. https://doi.org/10.3168/jds.2014-8695.

Guinee, T.P., E.O. Mulholland, J. Kelly, and D.J.O. Callaghan. 2007. Effect of protein-to-fat ratio of milk on the composition, manufacturing efficiency, and yield of cheddar cheese. J Dairy Sci 90:110–123. https://doi.org/10.3168/jds.S0022-0302(07)72613-9.

Hammami, H., J. Vandenplas, M.L. Vanrobays, B. Rekik, C. Bastin, and N. Gengler. 2015. Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows. J Dairy Sci 98:4956–4968. https://doi.org/10.3168/jds.2014-9148.

Hansen, B.G., and Y. Li. 2017. An Analysis of Past World Market Prices of Feed and Milk and Predictions for the Future. Agribusiness 33:175–193. https://doi.org/10.1002/agr.21474.

Hayes, E., D. Wallace, C. O'Donnell, D. Greene, D. Hennessy, N. O'Shea, J.T. Tobin, and M.A. Fenelon. 2023. Trend analysis and prediction of seasonal changes in milk composition from a pasture-based dairy research herd. J Dairy Sci 106:2326–2337. https://doi.org/10.3168/jds.2021-21483.

Koukkari, W.L.., and R.B.. Sothern. 2007. Introducing Biological Rhythms : A Primer on the Temporal Organization of Life, with Implications for Health, Society, Reproduction, and the Natural Environment. 1st edition. Springer Netherlands, Dordrecht, The Netherlands.

Li, S., A. Ye, and H. Singh. 2019. Seasonal variations in composition, properties, and heat-induced changes in bovine milk in a seasonal calving system. J Dairy Sci 102:7747–7759. https://doi.org/10.3168/jds.2019-16685.

Lincoln, G.A., I.J. Clarke, R.A. Hut, and D.G. Hazlerigg. 2006. Characterizing a mammalian circannual pacemaker. Science (1979) 314:1941–1944. https://doi.org/10.1126/science.1132009.

Macciotta, N.P.P., D. Vicario, and A. Cappio-Borlino. 2005. Detection of different shapes of lactation curve for milk yield in dairy cattle by empirical mathematical models. J Dairy Sci 88:1178–1191. https://doi.org/10.3168/jds.S0022-0302(05)72784-3.

Marcondes, M.I., D.C. Jácome, A.L. da Silva, L.N. Rennó, and A.C. dos S. Pires. 2014. Evaluation of raw milk quality in different production systems and periods of the year. Revista Brasileira de Zootecnia 43:670–676. https://doi.org/10.1590/S1516-35982014001200007.

Nakagawa, S., P.C.D. Johnson, and H. Schielzeth. 2017. The coefficient of determination R2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. J R Soc Interface 14. https://doi.org/10.1098/rsif.2017.0213.

Nightingale, C., K. Dhuyvetter, R. Mitchell, and Y. Schukken. 2008. Influence of variable milk quality premiums on observed milk quality. J Dairy Sci 91:1236–1244. https://doi.org/10.3168/jds.2007-0609.

Niu, M., Y. Ying, P.A. Bartell, and K.J. Harvatine. 2014. The effects of feeding time on milk production, total-tract digestibility, and daily rhythms of feeding behavior and plasma metabolites and hormones in dairy cows. J Dairy Sci 97:7764–7776. https://doi.org/10.3168/jds.2014-8261.

Nogara, K.F., G.C.B. Kaelle, Q.G. Tavares, T.R. Marcon, E. Gopinger, M. Zopollatto, and E. de Camargo Debortoli. 2022. Influence of the seasons on the quality and composition of milk from dairy farms in the northern region of Rio Grande do Sul, Brazil. Ciencia Animal Brasileira 23. https://doi.org/10.1590/1809-6891v23e-72795P.

Noro, G., F. Hilario Diaz González, R. Campos, and J. Walter Dürr. 2006. Fatores ambientais que afetam a produção e a composição do leite em rebanhos assistidos por cooperativas no Rio Grande do Sul. 1129–1135.

Olde Riekerink, R.G.M., H.W. Barkema, and H. Stryhn. 2007. The effect of season on somatic cell count and the incidence of clinical mastitis. J Dairy Sci 90:1704–1715. https://doi.org/10.3168/jds.2006-567.

Olipra, J. 2019. Change in Seasonality Pattern of EU Farmgate Milk Price. Zeszyty Naukowe SGGW w Warszawie - Problemy Rolnictwa Światowego 19:75–84. https://doi.org/10.22630/prs.2019.19.3.48.

Palmquist, D.L., A. Denise Beaulieu, and D.M. Barbano. 1993. Feed and Animal Factors Influencing Milk Fat Composition. J Dairy Sci 76:1753–1771. https://doi.org/10.3168/jds.S0022-0302(93)77508-6.

Piccioli-Cappelli, F., J.J. Loor, C.J. Seal, A. Minuti, and E. Trevisi. 2014. Effect of dietary starch level and high rumen-undegradable protein on endocrine-metabolic status, milk yield, and milk composition in dairy cows during early and late lactation. J Dairy Sci 97:7788–7803. https://doi.org/10.3168/jds.2014-8336.

Piccione, G., V. Messina, S. Scianó, A. Assenza, T. Orefi Ce, I. Vazzana, and A. Zumbo. 2012. Annual changes of some metabolical parameters in dairy cows in the Mediterranean area. Vet Arh 82:229–238.

Poppe, M., H.A. Mulder, and R.F. Veerkamp. 2021. Validation of resilience indicators by estimating genetic correlations among daughter groups and with yield responses to a heat wave and disturbances at herd level. J Dairy Sci 104:8094–8106. https://doi.org/10.3168/jds.2020-19817.

R Core Team. 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Salfer, I.J., P.A. Bartell, C.D. Dechow, and K.J. Harvatine. 2020. Annual rhythms of milk synthesis in dairy herds in 4 regions of the United States and their relationships to environmental indicators. J Dairy Sci 103:3696–3707. https://doi.org/10.3168/jds.2019-17362.

Salfer, I.J., C.D. Dechow, and K.J. Harvatine. 2019. Annual rhythms of milk and milk fat and protein production in dairy cattle in the United States. J Dairy Sci 102:742–753. https://doi.org/10.3168/jds.2018-15040.

Sicheski, S.J., I.M.P. Haygert-Velho, M.A. Piuco, M. Busanello, J.L.B. Calgaro, P.S.G. Almeida, J.M. Da Conceição, and J.P. Velho. 2020. Longitudinal retrospective study on the effect of season on milk production and composition in Rio Grande do Sul, Brazil. Semina:Ciencias Agrarias 41:1355–1372. https://doi.org/10.5433/1679-0359.2020v41n4p1355.

Silpa, M.V., S. König, V. Sejian, P.K. Malik, M.R.R. Nair, V.F.C. Fonseca, A.S.C. Maia, and R. Bhatta. 2021. Climate-Resilient Dairy Cattle Production: Applications of Genomic Tools and Statistical Models. Front Vet Sci 8. https://doi.org/10.3389/fvets.2021.625189.

West, J.W. 2003. Effects of heat-stress on production in dairy cattle. J Dairy Sci 86:2131–2144. https://doi.org/10.3168/jds.S0022-0302(03)73803-X.

Wheelock, J.B., R.P. Rhoads, M.J. VanBaale, S.R. Sanders, and L.H. Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J Dairy Sci 93:644–655. https://doi.org/10.3168/jds.2009-2295.

Yang, L., Q. Yang, M. Yi, Z.H. Pang, and B.H. Xiong. 2013. Effects of seasonal change and parity on raw milk composition and related indices in Chinese Holstein cows in northern China. J Dairy Sci 96:6863–6869. https://doi.org/10.3168/jds.2013-6846.

Tables and figures

Table 1. The amplitude and acrophase of a cosine function with a 12-mo period fit to the state-level monthly averages of milk fat, protein, and somatic cell count concentration

Item	State	N	Mean	Amplitude[1]	Acrophase, date[2]	P-value[3]
Fat, %	Goiás	132	3.601[a]	0.184[a]	Apr 26[a]	< 0.001
	Minas Gerais	132	3.641[b]	0.167[b]	May 10[b]	< 0.001
	Paraná	132	3.744[c]	0.157[c]	May 12[b]	< 0.001
	Rio Grande do Sul	132	3.843[d]	0.152[c]	May 12[b]	< 0.001
	Santa Catarina	132	3.969[e]	0.163b	May 09[b]	< 0.001
	São Paulo	132	3.617[f]	0.192[a]	May 11[b]	< 0.001
Protein, %	Goiás	132	3.274[a]	0.097[a]	Mar 25[a]	< 0.001
	Minas Gerais	132	3.251[b]	0.094[a]	Apr 08[b]	< 0.001
	Paraná	132	3.239[c]	0.088[b]	May 07[c]	< 0.001
	Rio Grande do Sul	132	3.255[b]	0.102[a]	May 30[d]	< 0.001
	Santa Catarina	132	3.264[d]	0.088[b]	May 20[e]	< 0.001
	São Paulo	132	3.249[b]	0.095[a]	Apr 08[b]	< 0.001
Somatic cell count, $\times 10^3$ cell/mL	Goiás	132	369.6[a]	18.6[a]	Jan 05[a]	< 0.001
	Minas Gerais	132	449.6[b]	24.1[b]	Jan 13[b]	< 0.001
	Paraná	132	436.7[c]	22.3[c]	Feb 19[c]	< 0.001
	Rio Grande do Sul	132	553.4[d]	33.0[d]	Feb 10[d]	< 0.001
	Santa Catarina	132	486.5[e]	33.0[d]	Feb 23[e]	< 0.001
	São Paulo	132	394.5[f]	16.4[e]	Jan 14[b]	< 0.001

[a–f] Values that do not share a superscript differ ($P < 0.05$). State-specific mesor, amplitude, and acrophase were compared using pairwise contrasts of model-predicted values with Tukey adjustment ($P < 0.05$).

[1] Distance from the mean to the peak of the 12-mo rhythm. Amplitude is expressed in % for fat and protein, and in $\times 10^3$ cells/mL for SCC.

[2] Date at peak of the 12-mo fitted rhythm as month and day of month.

[3] P-value for the zero-amplitude test corresponding to the fit of a 12-mo cosine rhythm.

Table 2. Performance of regression models with state fixed effects for predicting milk protein content

Model[1]	Fixed effects[2]	n	R^2	AIC	BIC	RMSE
12	state + cos12 + sin12 + soy	792	0.654	-2682.3	-2630.9	0.0404
01	state + cos12 + sin12	792	0.653	-2700.8	-2654.1	0.0404
14	state + cos12 + sin12 + soy + corn	792	0.653	-2667.7	-2611.6	0.0404
11	state + cos12 + sin12 + milk_brazil	792	0.647	-2690.5	-2639.1	0.0404
13	state + cos12 + sin12 + corn	792	0.642	-2683.7	-2632.3	0.0403
15	state + cos12 + sin12 + soy + corn + milk_state	792	0.631	-2663.7	-2602.9	0.0401
10	state + cos12 + sin12 + milk_state	792	0.614	-2695.0	-2643.6	0.0401
03	state + soy	792	0.237	-1805.9	-1763.9	0.0710
06	state + corn + soy	792	0.205	-1801.6	-1754.9	0.0707
08	state + soy + milk_state	792	0.198	-1835.3	-1788.5	0.0696
09	state + soy + corn + milk_state	792	0.138	-1837.2	-1785.8	0.0693
05	state + milk_brazil	792	0.073	-1817.3	-1775.2	0.0726
07	state + corn + milk_state	792	0.068	-1802.2	-1755.4	0.0726
04	state + milk_state	792	0.064	-1813.2	-1771.1	0.0728
02	state + corn	792	0.026	-1793.4	-1751.3	0.0730

[1] Models ranked by R^2 in descending order. R^2 marginal values represent the proportion of variance explained by fixed effects alone, calculated using the method of Nakagawa et al. (2017).

[2] Cosinor terms (cos12 and sin12) represent the annual (12-mo) seasonal oscillation. State indicates the fixed effect of the federative unit. Soy and Corn represent monthly soybean and corn prices, respectively (BRL/kg). Milk_state corresponds to the average monthly milk price at the state level, and milk_brazil to the national average milk price.

[3] All price data were obtained from the Center for Advanced Studies on Applied Economics (CEPEA), Luiz de Queiroz College of Agriculture, University of São Paulo (Piracicaba, Brazil).

[4] All models were fitted as national linear mixed-effects regressions with state as a fixed effect and year as a random effect, using the lmer() function from the lme4 package (R version 4.5.1).

Table 3. Performance of regression models with state fixed effects for predicting milk fat content

Model[1]	Fixed effects[2]	n	R^2	AIC	BIC	RMSE
01	state + cos12 + sin12	792	0.935	-2496.2	-2449.5	0.0469
10	state + cos12 + sin12 + milk_state	792	0.935	-2484.8	-2433.4	0.0469
11	state + cos12 + sin12 + milk_brazil	792	0.935	-2484.8	-2433.4	0.0469
12	state + cos12 + sin12 + soy	792	0.935	-2476.9	-2425.5	0.0469
14	state + cos12 + sin12 + soy + corn	792	0.935	-2465.3	-2409.2	0.0468
15	state + cos12 + sin12 + soy + corn + milk_state	792	0.935	-2456.4	-2395.7	0.0467
13	state + cos12 + sin12 + corn	792	0.934	-2477.4	-2426.0	0.0469
09	state + soy + corn + milk_state	792	0.573	-1001.2	-949.7	0.1187
08	state + soy + milk_state	792	0.571	-1012.3	-965.5	0.1188
07	state + corn + milk_state	792	0.548	-964.5	-917.8	0.1233
04	state + milk_state	792	0.524	-945.9	-903.8	0.1275
05	state + milk_brazil	792	0.524	-946.0	-903.9	0.1275
02	state + corn	792	0.520	-932.5	-890.4	0.1280
03	state + soy	792	0.520	-931.5	-889.4	0.1280
06	state + corn + soy	792	0.520	-917.2	-870.4	0.1279

[1] Models ranked by R^2 in descending order. R^2 marginal values represent the proportion of variance explained by fixed effects alone, calculated using the method of Nakagawa et al. (2017).

[2] Cosinor terms (cos12 and sin12) represent the annual (12-mo) seasonal oscillation. State indicates the fixed effect of the federative unit. Soy and corn represent monthly soybean and corn prices, respectively (BRL/kg). Milk_state corresponds to the average monthly milk price at the state level, and milk_brazil to the national average milk price.

[3] All price data were obtained from the Center for Advanced Studies on Applied Economics (CEPEA), Luiz de Queiroz College of Agriculture, University of São Paulo (Piracicaba, Brazil).

[4] All models were fitted as national linear mixed-effects regressions with state as a fixed effect and year as a random effect, using the lmer() function from the lme4 package (R version 4.5.1).

Table 4. Comparison of best-performing versus cosinor-only models for milk protein concentration across Brazilian states

State	Model[1]	Fixed effects[2]	R^2	RMSE	AIC
Goiás	01	cos12 + sin12	0.795	0.0318	-482.0
Minas Gerais	14	cos12 + sin12 + soy + corn	0.861	0.0245	-516.0
	01	cos12 + sin12	0.850	0.0249	-542.9
Paraná	12	cos12 + sin12 + soy	0.802	0.0238	-524.7
	01	cos12 + sin12	0.756	0.0234	-540.5
Rio Grande do Sul	10	cos12 + sin12 + milk_state	0.762	0.0276	-490.1
	01	cos12 + sin12	0.701	0.0272	-497.3
Santa Catarina	11	cos12 + sin12 + milk_brazil	0.824	0.0258	-521.7
	01	cos12 + sin12	0.770	0.0251	-526.7
São Paulo	14	cos12 + sin12 + soy + corn	0.811	0.0299	-465.8
	01	cos12 + sin12	0.805	0.0301	-495.8

[1] For each state, the best-performing model selected by highest marginal R^2 is compared with the cosinor-only baseline (Model 01). R^2 represents marginal R^2 calculated using Nakagawa's method, indicating variance explained by fixed effects only.

[2] cos12 + sin12 = cosinor terms for 12-mo seasonal rhythm; soy = soybean price; corn = corn price; milk_state = state-level milk price; and milk_brazil = national average milk price (all prices in BRL/kg).

[3] All within-state models were fitted using linear mixed-effects regression with year as random effect using lmer() function from lme4 package. R^2 represents marginal R^2 calculated using Nakagawa's method. Analyses were performed in R version 4.5.1 (R Core Team, 2024).

Table 5. Comparison of best-performing versus cosinor-only models for milk fat concentration across Brazilian states

State	Model	Fixed effects	R^2	RMSE	AIC
Goiás	13	cos12 + sin12 + corn	0.916	0.0383	-425.9
	01	cos12 + sin12	0.901	0.0389	-428.5
Minas Gerais	10	cos12 + sin12 + milk_state	0.933	0.0303	-491.3
	01	cos12 + sin12	0.929	0.0297	-498.9
Paraná	15	cos12 + sin12 + soy + corn + milk_state	0.882	0.0400	-395.7
	01	cos12 + sin12	0.880	0.0408	-433.2
Rio Grande do Sul	10	cos12 + sin12 + milk_state	0.820	0.0482	-370.4
	01	cos12 + sin12	0.792	0.0477	-371.4
Santa Catarina	11	cos12 + sin12 + milk_brazil	0.865	0.0444	-395.6
	01	cos12 + sin12	0.844	0.0439	-395.3
São Paulo	15	cos12 + sin12 + soy + corn + milk_state	0.918	0.0403	-393.7
	01	cos12 + sin12	0.896	0.0390	-421.3

[1] For each state, the best-performing model selected by highest marginal R^2 is compared with the cosinor-only baseline (Model 01). R^2 represents marginal R^2 calculated using Nakagawa's method, indicating variance explained by fixed effects only.

[2] cos12 + sin12 = cosinor terms for 12-mo seasonal rhythm; soy = soybean price; corn = corn price; milk_state = state-level milk price; and milk_brazil = national average milk price (all prices in BRL/kg).

[3] All within-state models were fitted using linear mixed-effects regression with year as random effect using lmer() function from lme4 package. R^2 represents marginal R^2 calculated using Nakagawa's method. Analyses were performed in R version 4.5.1 (R Core Team, 2024).

Table 6. Herd-level cosinor summary of annual rhythms in milk fat, protein, somatic cell count, and milk yield for Girolando herds (41 herds; mean 18 cows/herd)

Item	Herds with significant rhythm, no. (%)[1]	Mean amplitude[2]	Amplitude CV, %[3]	Mean acrophase date[4]	Circular concentration[5]	R^2 (conditional)[6]
Fat, %	13 (31.7)	0.147	59.8	Jun 14	0.41	0.273
Protein, %	27 (65.9)	0.116	54.4	May 30	0.65	0.380
Somatic cell count, $\times 10^3$ cells/mL	9 (22.0)	253.7	86.9	Oct 16	0.09	0.344
Milk yield, kg/d	14 (34.1)	2.05	71.5	Aug 04	0.29	0.337

[1] Herds with a significant annual rhythm based on a zero-amplitude F-test comparing a 12-mo cosinor model (Y ~ cos12 + sin12) with an intercept-only model ($P < 0.05$).
[2] Distance from the herd-level mean (mesor) to the peak of the fitted 12-mo cosine function, expressed in the same units as the trait.
[3] Coefficient of variation of the amplitude, calculated as 100 × SD / mean across herds.
[4] Calendar date corresponding to the circular mean of herd-level acrophases (month and day).
[5] Resultant vector length (0–1) for the distribution of herd-level acrophases; values closer to 1 indicate greater concentration of acrophases around the mean direction.
[6] Conditional R^2 values represent the proportion of variance explained by both fixed and random effects, calculated using the method of Nakagawa et al. (2017).

Table 7. Herd-level cosinor parameters by state for Girolando herds

Item	State	N	Herds with significant rhythm, no. (%)[1]	Mean amplitude[2]	Mean acrophase[3]	R^2 (conditional)[4]
Fat, %	Minas Gerais	26	8 (30.8)	0.136	Jun 05	0.240
	Paraná	5	2 (40.0)	0.127	Jun 06	0.310
	São Paulo	10	3 (30.0)	0.185	Jul 05	0.366
Protein, %	Minas Gerais	26	17 (65.4)	0.115	May 26	0.385
	Paraná	5	3 (60.0)	0.087	May 26	0.360
	São Paulo	10	7 (70.0)	0.132	Jun 09	0.382
Somatic cell count, $\times 10^3$ cells/mL	Minas Gerais	26	7 (26.9)	242.496	Sep 27	0.326
	Paraná	5	1 (20.0)	160.533	Oct 01	0.351
	São Paulo	10	1 (10.0)	329.352	Jan 01	0.389
Milk yield, kg/d	Minas Gerais	26	8 (30.8)	1.923	Aug 09	0.346
	Paraná	5	1 (20.0)	1.481	Oct 08	0.267
	São Paulo	10	5 (50.0)	2.674	Nov 09	0.358

[1] Herds with a significant annual rhythm based on a zero-amplitude F-test comparing a 12-mo cosinor model ($Y \sim \cos12 + \sin12$) with an intercept-only model ($P < 0.05$).

[2] Distance from the herd-level mean (mesor) to the peak of the fitted 12-mo cosine function.

[3] Calendar date corresponding to the circular mean of the herd-level acrophases (month–day).

[4] Conditional R^2 values represent the proportion of variance explained by fixed and random effects, calculated according to Nakagawa et al. (2017), and are presented as the mean conditional R^2 across herds with significant rhythms within each state.

Table 8. Comparison of herd-level cosinor parameters by state for Girolando herds (2013–2023). Parameters were obtained from a single mixed-effects cosinor model fitted jointly across states, including fixed effects of state and 12-mo cosine and sine terms and random intercepts for herd and year

Item	State	N	Mesor (herd)	Amplitude (herd)[1]	Acrophase (month–day)[2]	P-value[3]	R^2 (conditional)[4]
Fat, %	Minas Gerais	26	3.7151	0.0638	Jun 09	0.013	0.186
	Paraná	5	3.8144	0.0287	Jun 27	0.013	0.186
	São Paulo	10	3.8066	0.0979	Jul 04	0.013	0.186
Protein, %	Minas Gerais	26	3.3678	0.0436	May 10	<0.001	0.228
	Paraná	5	3.3327	0.0587	May 24	<0.001	0.228
	São Paulo	10	3.2977	0.0612	Jun 10	<0.001	0.228
Somatic cell count, $\times 10^3$ cells/mL	Minas Gerais	26	620.90	130.05	Jul 12	0.061	0.274
	Paraná	5	757.91	74.17	Sep 22	0.061	0.274
	São Paulo	10	1062.99	173.07	Oct 15	0.061	0.274

[1] Distance from the mesor to the peak of the fitted 12-mo cosine function, expressed in percentage units (%) for fat and protein, and in $\times 10^3$ cells/mL for somatic cell count.

[2] Calendar date of the herd-level acrophase derived from the state-specific cosine and sine coefficients of the combined herd–state model.

[3] P-value from a global zero-amplitude test implemented as a likelihood-ratio test comparing the full 12-mo cosinor model (cos12 + sin12) with an intercept-only model. Because the validation model was fitted jointly across states for each trait, a single P-value is obtained per trait and therefore appears identical across states.

[4] Conditional R^2 from the combined mixed-effects herd–state validation model (Nakagawa et al., 2017). As R^2 is calculated for the global model rather than by state, a single conditional R^2 value is reported per trait and repeated across states; between-state differences are reflected in the mesor, amplitude, and acrophase estimates.

1085

Table 9. Cow-level cosinor summary of annual rhythms in milk fat, protein, somatic cell count, and milk yield for Girolando cows (41 herds; mean 18 cows/herd; mean 11.2 mo of records per cow).

Item	Cows with significant rhythm, no. (%)[1]	Mean amplitude[2]	Amplitude CV, %[3]	Mean acrophase date[4]	Circular concentration[5]	R^2 (adjusted)[6]
Fat, %	147 (20.5)	0.350	59.6	Jun 19	0.21	0.213
Protein, %	331 (46.2)	0.215	53.2	Jun 21	0.24	0.309
Somatic cell count, $\times 10^3$ cells/mL	159 (22.2)	462.037	120.9	Oct 15	0.07	0.216
Milk yield, kg/d	370 (51.6)	5.472	57.2	Jul 08	0.08	0.321

[1] Cows with a significant annual rhythm based on a zero-amplitude F-test comparing a 12-mo cosinor model (Y ~ cos12 + sin12) with an intercept-only model ($P < 0.05$).
[2] Distance from the cow-level mean (mesor) to the peak of the fitted 12-mo cosine function, expressed in the same units as the trait.
[3] Coefficient of variation of the amplitude, calculated as 100 × SD / mean across cows with significant rhythms.
[4] Calendar date corresponding to the circular mean of cow-level acrophases (month and day).
[5] Resultant vector length (0–1) for the distribution of cow-level acrophases; values closer to 1 indicate greater concentration of acrophases around the mean direction.
[6] Values represent the mean adjusted R^2 of individual cow-level cosinor models.

Table 10. Cow-level cosinor parameters by state for Girolando cows.

Item	State	N	Cows with significant rhythm, no. (%)[1]	Mean amplitude[2]	Mean acrophase[3]	R^2 (adjusted)[4]
Fat, %	Minas Gerais	475	94 (19.8)	0.599	Jul 09	0.214
	Paraná	102	23 (22.5)	0.456	Aug 12	0.192
	São Paulo	139	30 (21.6)	0.576	Jul 01	0.223
Protein, %	Minas Gerais	476	226 (47.5)	0.293	Jun 12	0.325
	Paraná	102	46 (45.1)	0.265	Jul 19	0.260
	São Paulo	139	59 (42.4)	0.287	Jun 09	0.326
Somatic cell count, $\times 10^3$ cells/mL	Minas Gerais	476	109 (22.9)	677.449	Oct 28	0.220
	Paraná	102	22 (21.6)	512.876	Oct 15	0.208
	São Paulo	139	28 (20.1)	718.172	Sep 22	0.214
Milk yield, kg/d	Minas Gerais	476	236 (49.6)	9.471	Jul 27	0.328
	Paraná	102	53 (52.0)	7.120	Jul 25	0.272
	São Paulo	139	81 (58.3)	6.560	Jul 22	0.322

[1] Cows with a significant annual rhythm based on a zero-amplitude F-test comparing a 12-mo cosinor model ($Y \sim \cos12 + \sin12$) with an intercept-only model ($P < 0.05$).

[2] Distance from the cow-level mean (mesor) to the peak of the fitted 12-mo cosine function, expressed in percentage units (%) for fat and protein, in $\times 10^3$ cells/mL for somatic cell count, and in kg/d for milk yield.

[3] Calendar date corresponding to the circular mean of cow-level acrophases within each state (month–day).

[4] R^2 values represent the mean adjusted R^2 across individual cow-level cosinor models within each state.

Table 11. Comparison of cow-level cosinor parameters by state for Girolando cows (2013–2023). Parameters were obtained from a single mixed-effects cosinor validation model fitted jointly across states, including fixed effects of state and 12-mo cosine and sine terms and random intercepts for cow, herd, and year

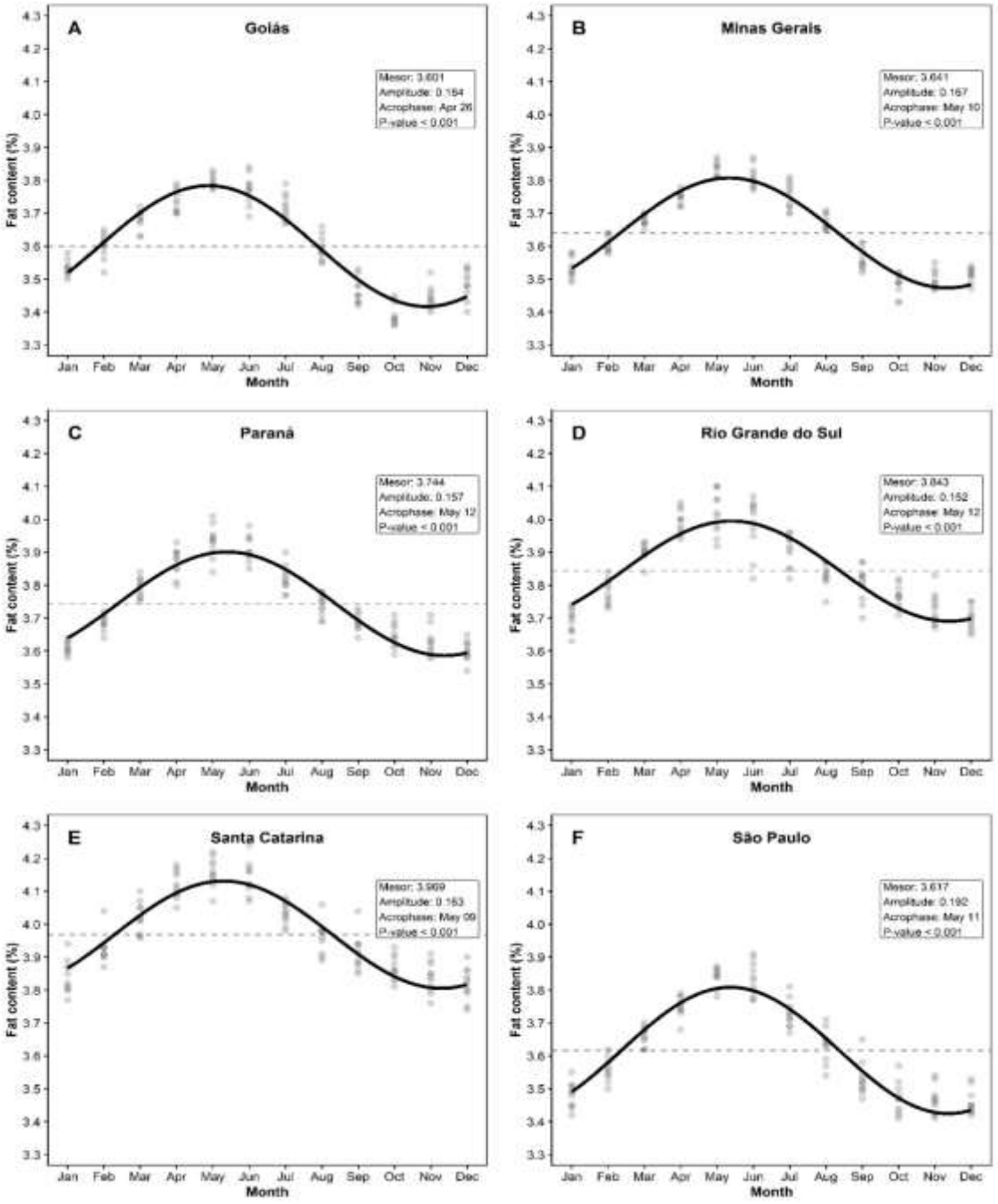
Item	State	N	Mesor (cow-level)	Amplitude (cow-level)[1]	Acrophase (month–day)[2]	P-value[3]	R^2 (conditional)[4]
Fat, %	Minas Gerais	475	3.7257	0.0860	Jun 06	<0.001	0.248
	Paraná	102	3.8233	0.0579	Jun 28	<0.001	0.248
	São Paulo	139	3.8003	0.1212	Jun 26	<0.001	0.248
Protein, %	Minas Gerais	476	3.3749	0.0565	May 19	<0.001	0.326
	Paraná	102	3.3764	0.0681	Jun 13	<0.001	0.326
	São Paulo	139	3.3047	0.0815	Jun 26	<0.001	0.326
Somatic cell count, $\times 10^3$ cells/mL	Minas Gerais	476	443.81	27.35	Oct 29	0.903	0.389
	Paraná	102	758.34	9.67	Jan 15	0.903	0.389
	São Paulo	139	933.26	18.36	Oct 01	0.903	0.389

[1] Distance from the cow-level mesor to the peak of the fitted 12-mo cosine function, expressed in percentage units (%) for fat and protein and in $\times 10^3$ cells/mL for somatic cell count.

[2] Calendar date of the cow-level acrophase estimated from the state-specific cosine and sine coefficients of the combined cow-level mixed-effects model.

[3] P-value from a global zero-amplitude test implemented as a likelihood-ratio test comparing the full 12-mo cosinor model (cos12 + sin12) with an intercept-only model. Because the validation model was fitted jointly across states for each trait, a single P-value is obtained per trait and therefore appears identical across states.

[4] Conditional R^2 from the combined cow-level mixed-effects state validation model (Nakagawa et al., 2017). As R^2 is calculated for the global model rather than by state, a single conditional R^2 value is reported per trait and repeated across states; between-state differences are reflected in the mesor, amplitude, and acrophase estimates.



X'

Figure 1. Annual rhythms of milk fat content in bulk tank milk from different Brazilian states: (A) Goiás, (B) Minas Gerais, (C) Paraná, (D) Rio Grande do Sul, (E) Santa Catarina, and (F) São Paulo. Data points represent observed monthly averages. The solid black line depicts the fitted cosine function with a 12-mo period, and the horizontal dashed line represents the mesor (rhythm-adjusted mean). Data were obtained from the Brazilian Milk Quality Observatory (PNQL/MAPA) based on analyses of bulk tank milk samples collected by laboratories of the Brazilian Milk Quality Network (RBQL). The mesor, amplitude (Amp; half of the peak-to-trough difference), acrophase (Acro; time at peak), and P-value for the zero-amplitude test are shown in each panel.

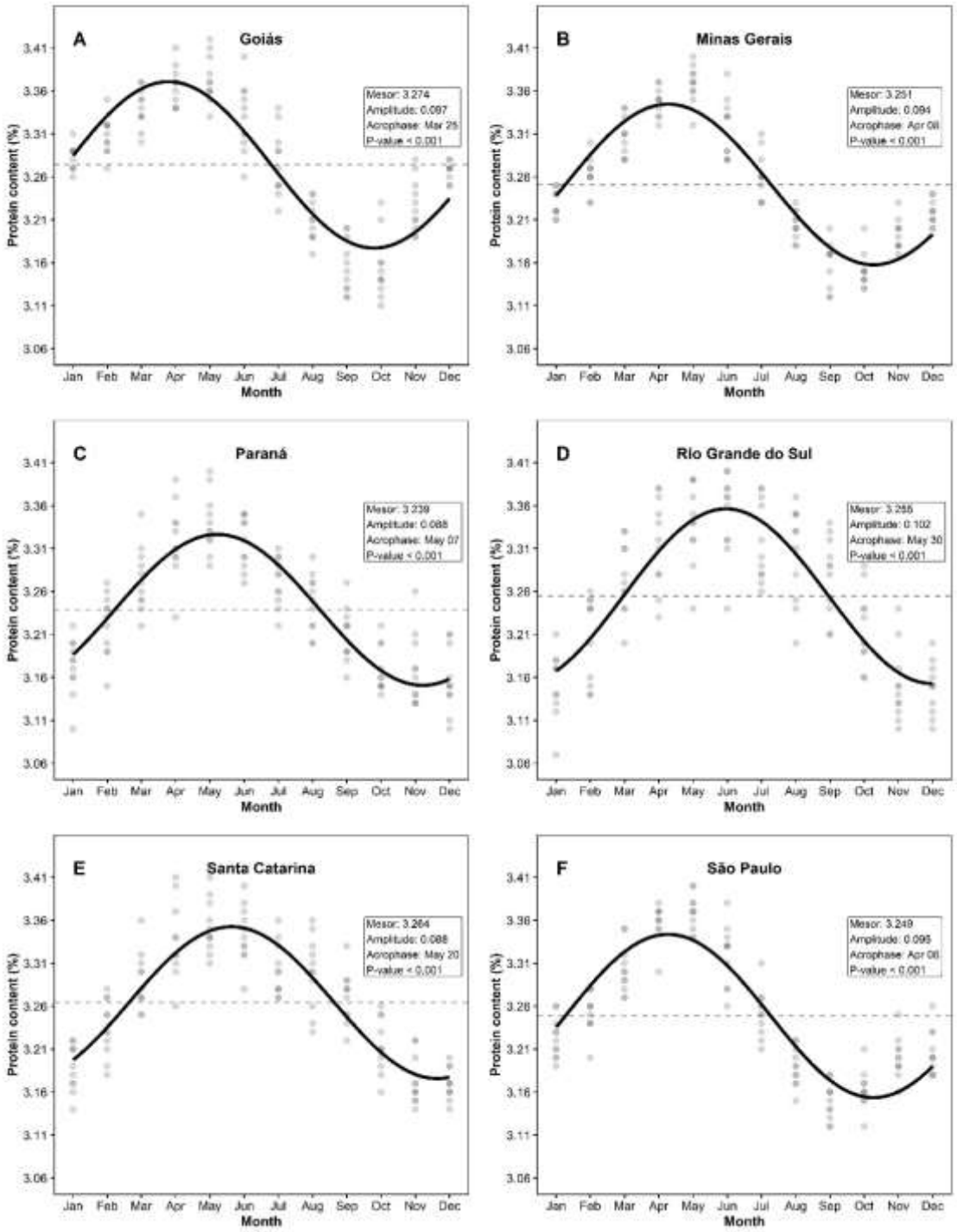


Figure 2. Annual rhythms of milk protein content in bulk tank milk from different Brazilian states: (A) Goiás, (B) Minas Gerais, (C) Paraná, (D) Rio Grande do Sul, (E) Santa Catarina, and (F) São Paulo. Data points represent observed monthly averages. The solid black line depicts the fitted cosine function with a 12-mo period, and the horizontal dashed line represents the mesor (rhythm-adjusted mean). Data were obtained from the Brazilian Milk Quality Observatory (PNQL/MAPA) based on analyses of bulk tank milk samples collected by laboratories of the Brazilian Milk Quality Network (RBQL). The mesor, amplitude (Amp; half of the peak-to-trough difference), acrophase (Acro; time at peak), and P-value for the zero-amplitude test are shown in each panel.

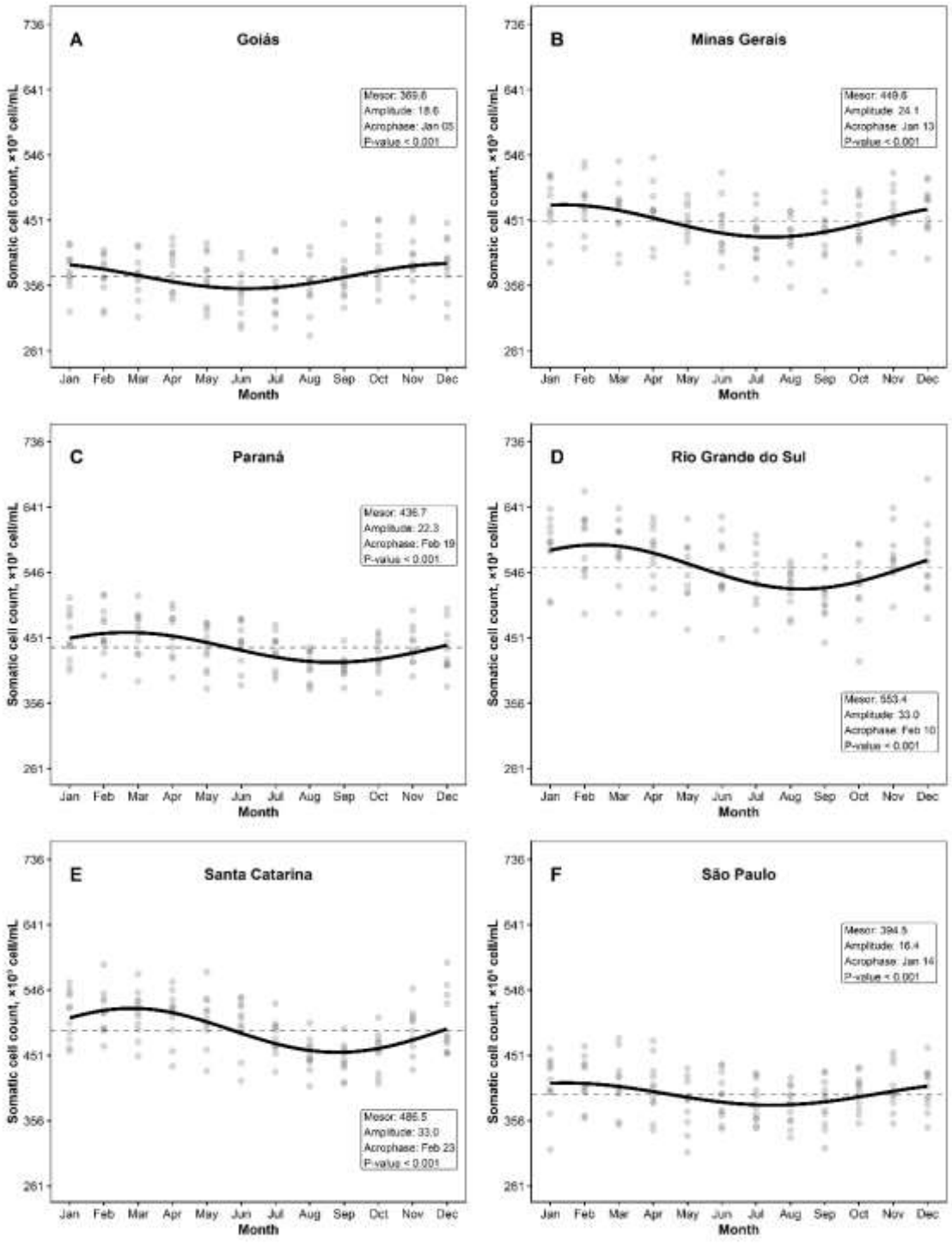


Figure 3. Annual rhythms of somatic cells count in bulk tank milk from different Brazilian states: (A) Goiás, (B) Minas Gerais, (C) Paraná, (D) Rio Grande do Sul, (E) Santa Catarina, and (F) São Paulo. Data points represent observed monthly averages. The solid black line depicts the fitted cosine function with a 12-mo period, and the horizontal dashed line represents the mesor (rhythm-adjusted mean). Data were obtained from the Brazilian Milk Quality Observatory (PNQL/MAPA) based on analyses of bulk tank milk samples collected by

laboratories of the Brazilian Milk Quality Network (RBQL). The mesor, amplitude (Amp; half of the peak-to-trough difference), acrophase (Acro; time at peak), and P-value for the zero-amplitude test are shown in each panel.

Artigo 2 - Redigido conforme norma do periódico científico - Versão preliminar	
Título do artigo:	Response of mid-lactation primiparous Holstein cows to the supplementation of rumen-protected methionine during the summer
Autores:	Caio Rodrigues Monteiro Victor Augusto de Oliveira Rabeche Schmith João Pedro Andrade Rezende Tales Lelis Resende João Alberto Negrão Marina de Arruda Camargo Danés
Periódico:	PloS One
ISSN	1932-6203

34

ARTIGO 2 – Response of mid-lactation primiparous Holstein cows to the supplementation of rumen-protected methionine during the summer

Caio R. Monteiro[1], Victor Augusto de Oliveira[1], Rabeche Schmith[1], João Pedro A. Rezende[1], Tales L. Resende[2], João A. Negrão[3], Marina A. C. Danés[1]*

[1] Department of Animal Science, Federal University of Lavras, Lavras, Minas Gerais, Brazil

[2] Evonik Brasil Ltda., São Paulo, São Paulo, Brazil

[3] Department of Basic Sciences, Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo (USP), Pirassununga, São Paulo, Brazil

***** Corresponding author

E-mail: marina.danes@ufla.br (MACD)

Abstract

This study aimed to evaluate the effects of rumen-protected methionine (RPM) supplementation on productive and physiological responses of primiparous Holstein cows during summer. We hypothesized that RPM supplementation would maintain or improve milk yield and composition due to beneficial physiological, redox, and inflammatory responses in cows exposed to summer heat. The trial was conducted in a randomized block design during nine weeks in Brazil using 80 primiparous cows (182 ± 64 DIM; 42.9 ± 4.7 kg/d milk). Cows were blocked by milk yield and DIM and assigned to a control diet (CON; no added RPM) or the same diet supplemented with RPM (Mepron®, Evonik) at 0.75 g/kg diet dry matter, targeting 20 g/cow/day (product contains 62% metabolizable methionine) to the average cow. Milk yield and composition, vaginal temperature, respiratory rate, and plasma samples were collected in weeks 3, 6, and 9. Data were analyzed using mixed models including treatment, week, and their interaction as fixed effects, and block and cow as random effects. Cows were maintained under naturally occurring summer conditions. Environmental monitoring during weeks 3, 6, and 9 indicated elevated temperature–humidity index (THI) values, with values remaining above the heat-stress threshold (THI > 68) for 68.3% of the monitored hours (mean THI = 70.6; range 61.0–84.4). Overall (least squares mean across weeks 3, 6, and 9), RPM increased milk yield by 2.0 kg/d (44.9 vs. 42.9 kg/d), protein yield by 50 g/d (1,464 vs. 1,414 g/d), lactose yield by 108 g/d (2,109 vs. 2,001 g/d), and total solids yield by 176 g/d (5,331 vs. 5,155 g/d). Lactose concentration was lower in RPM (4.71 vs. 4.76%). Fat yield was unaffected, but a treatment × week interaction was observed for fat content. Milk fatty acid (FA) profile was unchanged, although treatment × week interactions were observed for individual fatty acids (C16:0, C18:0, C18:1, and preformed FA). Plasma glucose was lower, and insulin was higher in RPM than in CON cows (39.3 vs. 43.2 mg/dL and 0.52 vs. 0.35 ng/mL, respectively). Antioxidant capacity improved, with RPM cows having higher ferric reducing antioxidant

power (32.9 vs. 28.5 µM) and lower malondialdehyde (2.48 vs. 2.78 nmol/mL). Other biochemical, inflammatory, and immune markers were unaffected. Respiratory rate was slightly higher in RPM than in CON cows (55 vs. 50 breaths/min). Mean vaginal temperature did not differ between treatments; however, a treatment × time × hour interaction was observed. Supplementation with RPM improved milk and solids yield, and enhanced antioxidant capacity and insulin levels, supporting its use to improve metabolic resilience under warm conditions.

Introduction

Heat stress (HS) is a well-recognized challenge that negatively affects the performance, health, and reproduction of dairy cows. Elevated temperatures are also associated with higher incidence of metabolic and infectious diseases and with impaired fetal development, potentially affecting future productive performance [1,2]. The ongoing global warming trend has intensified this issue, with climate models projecting a rise of approximately 0.2°C per decade in global mean temperature [3].

Although reduced dry matter intake (DMI) is considered the primary factor contributing to production losses during HS, it accounts for 35 to 50% of the observed decline in milk yield [4]. Additional physiological mechanisms, such as systemic inflammation, immune activation, and changes in the antioxidant system, contribute to the disruption of metabolic homeostasis [5,6]. These mechanisms alter nutrient partitioning, reducing the availability of key substrates for productive functions and increasing the diversion of amino acids (AA) toward nonproductive uses in the liver and gastrointestinal tract [7,8].

. Physical microclimate modifications (e.g., shade, increased air movement using fans, and evaporative cooling or cow-wetting strategies such as sprinklers/soakers or spray/misting) are widely adopted to enhance heat dissipation and mitigate heat stress in dairy cows [9,10,11]. In

parallel, nutritional strategies aimed at ameliorating the negative effects of heat stress have been investigated, but they are still less extensively characterized than mechanical cooling methods. These approaches include fat supplementation [12,13], vitamin and mineral supplementation [14,15], plant polyphenol extracts [16], antioxidant blends [17], and amino acid supplementation [18,19,20].

Among these, amino acids (AA) supplementation has emerged as a promising approach to support antioxidant capacity, modulate immune function, and maintain lactational performance. Methionine (Met) is frequently the most deficient AA in dairy cow diets and plays a central role not only in milk protein synthesis but also in one-carbon metabolism [21]. Under stress conditions, methionine is particularly important due to its involvement in methylation reactions, glutathione synthesis, and immune modulation [22].

Supplementation with rumen-protected methionine (RPM) during the transition period (from 3 wk prepartum to 3 wk postpartum) has been associated with improved lactational performance in multiparous Holstein cows. Feeding rumen-protected Met from −21 d to 30 DIM at 0.07–0.19% of diet DM increased milk yield by 3.4 kg/d and milk protein concentration by 0.18 percentage units [23], whereas ethyl-cellulose RPM fed from −28 to 60 d relative to parturition at 0.09–0.10% of diet DM increased milk yield by 4.1 kg/d and milk protein concentration by 0.16 percentage units during 1–30 DIM [24].

Continuous exposure to heat stress (HS) in dairy cows for one [25] to four [26] weeks can have cumulative negative impacts on production. In contrast, most studies evaluating rumen-protected methionine (RPM) under HS have relied on short-term challenge models lasting only a few days (e.g., a 9-d HS challenge), and therefore evidence on RPM supplementation during naturally occurring summer HS lasting several weeks, particularly in primiparous cows, remains limited. This knowledge gap is relevant because primiparous cows may be

128 moresusceptible under HS due to the simultaneous demands of growth, lactation, and metabolic
129 adaptation [27,28].

130 Therefore, the objective of this study was to evaluate the effects of RPM supplementation
131 on productive and physiological responses of primiparous Holstein cows during the summer.
132 We hypothesized that, despite both groups being managed under the same
133 mechanical/evaporative cooling system, RPM supplementation would maintain or improve
134 milk yield and composition, as well as support beneficial physiological, redox, and
135 inflammatory responses in lactating cows exposed to naturally occurring summer conditions in
136 Brazil.

137

138 Materials and methods

139 Ethics Statement

140 All procedures were approved by the Ethics Committee on the Use of Animals (CEUA) of
141 the Federal University of Lavras under protocol number 056/22.

142

143 Location, animals, management and facilities

144 The study was conducted on a commercial farm located in Três Corações, Minas Gerais,
145 in the Southeast region of Brazil, from December 2022 to February 2023. The farm is situated
146 at 910 m above sea level, at latitude 21°44'59.22"S and longitude 45°11'25.64"W.

147 A total of 80 primiparous Holstein cows with an average of 182 ± 64 days in milk (DIM),
148 producing 42.9 ± 4.7 kg of milk per day, with 2.93 ± 0.65% milk fat and 3.13 ± 0.27% milk
149 protein (mean ± SD) at the beginning of the experimental period were used. The animals were
150 housed in a compost barn with 120 other non-experimental cows, totaling 200 cows. They were

milked three times daily at 04:00, 12:00, and 20:00 in a herringbone parlor with automatic detachers.

The animals were housed in two pens measuring 16 × 50 m (800 m²) each, with 100 animals per pen (40 experimental and 60 non-experimental cows), resulting in a stocking density of 8.0 m² per cow. Each pen was assigned to one dietary treatment and was fed separately under commercial farm conditions; therefore, individual daily dry matter intake was not measured. Bedding consisted of wood shavings, mechanically turned twice daily. Both pens were equipped with four fans per pen (Mamute F200; FrontAgro Ltda., São José do Rio Preto, SP, Brazil) operating continuously and a sprinkler line along the feed bunk running on a 30 s on/4 min off cycle. The cooling system was managed identically in both pens throughout the experimental period.

Diets and experimental design

The experimental design was a randomized complete block design with two treatments. The 80 experimental cows were organized into 40 blocks (pairs) based on pre-trial milk production and DIM. Within each block, cows were randomly assigned to one of two treatments (one cow per treatment within each block): a control diet (CON) or CON plus the inclusion of RPM (Mepron®, Evonik Operations GmbH, Hanau, Germany), encapsulated with ethyl cellulose. At enrollment, cows were allocated to their respective pens and remained in the same pen throughout the experimental period.

A total of 40 cows per treatment were used, except for vaginal temperature, which was measured in a subsample of 15 cows per treatment. Sample size was determined using milk protein yield as the primary response variable, following Kononoff and Hanford [29] and adapted to the present design. Calculations were performed using a two-sided $\alpha = 0.05$ with a target power of 0.80. Based on farm records collected before the start of the experiment, the

standard deviation of milk protein yield was 94 g/d, and a 40 g/d difference was considered the minimum relevant effect to detect.

Experimental diets (Table 1) were formulated to meet or exceed requirements for metabolizable energy, metabolizable protein, vitamins, and macrominerals, as recommended by NASEM [30], to support milk production. The RPM dose was 0.75 g/kg of dietary dry matter (DM) in the total mixed ration (TMR; 0.075% of DM), corresponding to approximately 0.47 g of metabolizable methionine per kg of diet DM (≈0.047% of DM), based on 62% metabolizable methionine in the product, and to approximately 13 g of metabolizable methionine per cow per day. This dose was selected to increase the estimated metabolizable methionine supply and resulted in a methionine utilization efficiency below the target, indicating a nonlimiting (adequate-to-excess) supply of metabolizable methionine according to the NASEM ration evaluation [30]. The RPM product was pre-mixed into the mineral and vitamin supplement included in the TMR. The control diet contained no added rumen-protected methionine (0 g/kg DM of RPM). Baseline methionine supply was provided by the basal ingredients in both diets. Estimated metabolizable methionine supply and the Lys:Met ratio are shown in Table 1.

The experimental diets were provided as a TMR once daily at 08:00. Ingredients were weighed and mixed in a mixer wagon with an integrated scale (Siloking DUO 14-T®, Siloking do Brasil Ltda., São José do Rio Preto, São Paulo, Brazil). Refusals were weighed daily at the pen level, and the TMR amount was adjusted based on the average pen refusals from the previous two days, targeting 5% refusals. Animals had *ad libitum* access to TMR and fresh water. The TMR was pushed up with a motorized blade at least 10 times per day. Refusals were used only to manage feed delivery and were not used to estimate individual dry matter intake.

Due to the nature of the experimental design, blinding was not possible. The research team was aware of group allocation during animal assignment and the conduct of the experiment. Farm staff and researchers responsible for feeding and sampling were also aware of treatments.

For standardization, the 120 non-experimental cows were also paired by parity, milk production, and DIM and randomly assigned to one of the two pens. Thus, each pen contained 100 cows (40 experimental/sampled and 60 non-experimental/non-sampled) and was randomly assigned to one of the two treatments (CON or RPM).

Table 1. Ingredient and nutrient composition of control (CON) and rumen-protected methionine (RPM) supplemented diets[1].

	CON	RPM
Ingredient, % of DM		
Corn silage, main crop	41.0	41.0
Corn silage, second crop	11.1	11.1
Soybean meal	16.9	16.9
Citrus pulp	8.9	8.9
Corn, high-moisture silage	7.6	7.6
Distillers' dried grains	4.4	4.4
Pelletized soybean hulls	2.3	2.3
Cottonseed	4.2	4.2
Palm fat[2]	1.0	1.0
PREMIX[3]	2.5	2.5
Rumen-protected methionine[4]	---	0.075
Chemical composition, % of DM		
Crude protein	17.4	17.5
Metabolizable protein	10.0	10.1
Rumen-degradable protein	11.5	11.5
Rumen-undegradable protein	5.9	6.0
Starch	24.7	24.7
Neutral detergent fiber	29.3	29.3
Acid detergent fiber	17.7	17.7
Fatty acids	2.8	2.8
Ash	4.4	4.4
Metabolizable methionine, g/day[5]	55.0	68.0
Diet Lys:Met ratio[5]	3.2	2.5

[1]Diets were formulated to be isoenergetic and isonitrogenous.
[2]Palm fat: GoldenFat® (CSB Indústria e Comércio Ltda., Bragança Paulista, São Paulo, Brazil), composed of calcium salts of palm fatty acids. Guaranteed analysis: ether extract, minimum 700 g/kg; ash, maximum 190 g/kg; calcium, 100–110 g/kg.
[3]PREMIX: PEC-PRO Lactation Top 700 ORG (PEC-MIX Suplementos Minerais Ltda., Alfenas, Minas Gerais, Brazil). Guaranteed analysis: 9–14% Ca; 2.4% P; 11.4% Na; 3.1% Mg; 0.7% S; 1.7% K; 0.3% Zn; 0.2% Mn; 503 mg/kg Cu; 37 mg/kg I; 31.1 mg/kg Co; 17.8 mg/kg Se; 9.8 mg/kg Cr. Vitamins: A (220,657 IU/kg), D_3 (70,560 IU/kg), E (1,844 IU/kg). Additives: Monensin (475 mg/kg), Virginiamycin (490 mg/kg), Biotin (49 mg/kg), Bacillus subtilis (30×10^{11} CFU/kg), β-glucans (3,160 mg/kg), glucomannans (5,080 mg/kg), mannan-oligosaccharides (1,920 mg/kg), and calcium/sodium aluminosilicate (7,920 mg/kg).

[4]Rumen-protected methionine: Mepron® (Evonik Operations GmbH, Hanau, Germany), a rumen-protected DL-methionine product containing 85% DL-methionine.
[5]Estimated using NASEM (2021).

Data and sample collection and analysis

Before the start of the experiment and during experimental weeks 3, 6, and 9, TMR ingredients were sampled separately once daily. A weekly composite sample of each ingredient was stored frozen until the end of the trial. After completion of the experiment, composite samples were dried in a forced-air oven at 55°C for 72 h and ground to 1 mm in a Willey-type mill for analysis of dry matter [31], organic matter [31], ash [32], crude protein [32], ether extract [31], neutral detergent fiber (NDF) with amylase and sodium sulfite [33], acid detergent fiber [34], and starch [35]. Analyses were conducted at UFLA's Animal Production Laboratory, in Lavras, Minas Gerais, Brazil. The mean nutritional composition of the diet is presented in Table 1.

The trial lasted 9 weeks (63 days). The sampling weeks were defined as days 14–21 (week 3), 35–42 (week 6), and 56–63 (week 9) relative to the start of the experimental diets. During each sampling week, milk yield was recorded daily. Individual milk samples were collected on two consecutive days (days 19–20, 40–41, and 61–62, corresponding to the third-to-last and second-to-last day of each sampling week), and daily composite samples were prepared proportionally to the yield of each milking. Blood samples were collected on the last day of each sampling week (days 21, 42, and 63). Respiratory rate was recorded on two consecutive days during each sampling week (days 19–20, 40–41, and 61–62). Vaginal temperature was recorded continuously over four consecutive days corresponding to the last four days of each sampling week (days 18–21, 39–42, and 60–63). Mean (± SD) DIM on the last day of each sampling week (days 21, 42, and 63) was 203.6 ± 68.0, 224.6 ± 68.0, and 245.6 ± 68.0 for CON cows, and 201.6 ± 59.5, 222.6 ± 59.5, and 243.6 ± 59.5 for RPM cows, respectively.

Environmental conditions

Air temperature (°C) and relative humidity (%) were recorded automatically at 10-minute intervals inside the compost-barn by four automatic loggers (IP-747RH, Impac Comercial e Tecnologia Ltda., Vargem Grande Paulista, São Paulo, Brazil) positioned throughout the barn (two in each pen), 2 m above the floor. Temperature-humidity index (THI) was calculated at 1-hour intervals according to Mader et al. [36], using the mean ambient temperature and relative humidity recorded during each hour, as follows: $THI = (0.8\ x\ T) + [RH\ x\ (T - 14.4)] + 46.4$, where T is the ambient temperature (°C), and RH is the relative humidity expressed as a decimal fraction. The percentage of hours per day when THI exceeded 68 and 72 was then calculated. Across the entire experimental period, ambient temperature ranged from 16.1 to 33.0°C and THI ranged from 61.0 to 84.4. Environmental data are summarized in the Results section.

Vaginal temperature, and respiratory rate

Vaginal temperature (VT; °C) was measured in a subsample of 30 cows (15 per treatment) every five minutes over four consecutive days during weeks 3, 6, and 9 using an automatic temperature recorder (Thermochron iButton DS1922T-F5#, Analog Devices, Inc., Wilmington, MA, USA) with an accuracy of ±0.5°C and a measurement range of -10°C to +65°C. Temperature recorders were attached to an intravaginal device without progesterone (CIDR®; Zoetis, São Paulo, Brazil). Respiratory rate (RR) was measured by counting flank movements over 30 seconds for all experimental cows (n= 40) on two consecutive days during weeks 3, 6, and 9, starting at 10:00 and 17:00.

Milk collection and analysis of components

Milk production was measured on two consecutive days before the start of the experiment (baseline; days -2 and -1) and was included as a covariate in the statistical analyses of the corresponding outcomes (i.e., baseline milk yield for milk production traits, and baseline milk component values for milk composition traits). Milk production was also recorded daily during sampling weeks 3, 6, and 9 using an automatic meter (ACR Smart MMV, Interplus, Albinea, RE, Italy). Individual milk samples were collected before the start of the experiment (baseline) and during weeks 3, 6, and 9. Sampling was performed on two consecutive days (six milkings) during each sampling week (days 19–20, 40–41, and 61–62 relative to the start of the trial), at each milking (04:00, 12:00, and 20:00). A daily composite sample was prepared proportionally to the yield of each milking for each cow. Milk samples were stored in plastic vials with 2-bromo-2-nitropropane-1,3-diol (bronopol) preservative, homogenized, and kept refrigerated (4°C) until sent for analysis.

Individual daily milk samples were analyzed by mid-infrared Fourier-transform infrared spectroscopy (FTIR; CombiFoss 7, FOSS Analytics, Hillerød, Denmark) to determine fat, protein, lactose, casein, total solids. Milk fatty acids (FA) and FA groups (e.g., SFA, UFA, MUFA, PUFA; and chain-length classes) were predicted from FTIR spectra using the instrument's FA prediction models. Somatic cell count was determined by flow cytometry (MilkoscanCombiFoss 7, FOSS Analytics, Hillerød, Denmark). Analyses were conducted at the Clínica do Leite Institute Laboratory (Piracicaba, São Paulo, Brazil), without treatment identification. Daily milk solids yield was calculated using the milk yield and composition from the same day of sampling. Energy-corrected milk (ECM) was calculated using the equation: ECM = [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein yield (kg)], based on the week the milk was sampled [30].

The FTIR-based method quantified specific FA, including C14, C16, C18, C18:1, as well as broader categories such as saturated FA (SFA), unsaturated FA (UFA), monounsaturated FA (MUFA), and polyunsaturated FA (PUFA). Additionally, FA were also grouped according to their origin, comprising short-chain FA (C4 to C14) - de novo; medium chain FA (C15 and C16) - mixed; and long-chain fatty acids (C17 or more) - preformed. Daily yield of each milk fatty acid was calculated by multiplying its concentration by the daily milk yield.

Blood collection and analysis of metabolites

Blood samples were collected before the start of the experiment as a covariate and once during weeks 3, 6, and 9 by coccygeal vein puncture, always on the last day of the sampling week (days 21, 42, and 63), always after the second milking (starting at 12h15m) and approximately 4 h after feeding (TMR offered once daily at 08:00). Three samples per animal were collected: one for serum collection in a tube with clot activator, and two for plasma collection (one in a tube with sodium heparin anticoagulant and another in a tube with K3 EDTA). Samples were kept on ice and, after collection, centrifuged (3,000 rpm) for 30 minutes at room temperature to obtain serum or plasma and then frozen at -20°C in 2 mL microtubes for later analysis.

Serum samples were analyzed to assess nutritional status and liver health using commercial kits (all from Bioclin-Quibasa, Belo Horizonte, Minas Gerais, Brazil) and an automatic biochemical analyzer (SX-300, Sinnowa Brasil, Ribeirão Preto, São Paulo, Brazil). The analyses included albumin (K040-1), total cholesterol (K083-2), HDL cholesterol (K071-23), glucose (K-082-2,), urea nitrogen (K056-4.1), alanine aminotransferase (ALT; K049-6), and aspartate aminotransferase (AST; K048-6).

Oxidative status was assessed by analyzing plasma concentrations of ferric reducing antioxidant power (FRAP) using a commercial fluorometric kit (MAK509, Sigma-Aldrich,

Saint Louis, MO, USA) in samples collected during week 9. Concentrations of malondialdehyde [37], superoxide dismutase [38], and glutathione peroxidase [39] were determined in samples collected during the covariate period and weeks 3, 6, and 9.

Plasma concentrations of non-esterified fatty acids (NEFA) were assessed using an enzyme-linked immunosorbent assay (ELISA; FA115, Randox Laboratories Ltd., Crumlin, County Antrim, UK). Concentrations of haptoglobin and immunoglobulin A (IgA) were determined by polyacrylamide gel electrophoresis [40]. Hormonal response was evaluated using ELISA tests to measure plasma concentrations of insulin (3625-300A, Monobind Inc., Lake Forest, CA, USA) and cortisol (2425-300B, Monobind Inc., Lake Forest, CA, USA). All these analyses were performed using plasma samples collected only during week 9, when environmental temperatures were more challenging. Laboratory analyses performed by external facilities were conducted without treatment identification.

Statistical analysis

Statistical analyses were conducted using the MIXED procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). Response variables for blood parameters, milk production, and milk composition were evaluated using a randomized complete block with repeated measures over time, with pre-experiment collection values included as covariates in the model when baseline measurements of the same outcome were available. The model included week of sampling and treatment as fixed effects, and block, experimental unit (cow), and treatment-by-time interaction as random effects, as per the following model:

$$\boldsymbol{Y_{ijkl} = \mu + T_i + COY + W_l + B_j + V_k(B) + T\,x\,W_{il} + E_{ijkl}}$$

Where: $\boldsymbol{Y}$ = dependent variable; $\boldsymbol{\mu}$ = overall mean; $\boldsymbol{T_i}$ = fixed effect of treatment (i = CON or RPM); $\boldsymbol{COY}$ = covariate for $\boldsymbol{Y}$; $\boldsymbol{W_l}$ = fixed effect of week (l = 3, 6 or 9); $\boldsymbol{B_j}$ = random effect

of block (j = 1–40); $\boldsymbol{V_k(B)}$ = random effect of the experimental unit nested within block (k = 1-80); $\boldsymbol{T\ x\ W_{il}}$ = treatment-by-week interaction; and $\boldsymbol{E_{ijkl}}$ = residual error.

For blood metabolites that were measured only once during the experiment (week 9), such as haptoglobin, immunoglobulin A (IgA), insulin, cortisol, and ferric reducing antioxidant power (FRAP), the analysis was performed without including pre-experiment values as covariates or week as a repeated factor, because baseline values were not available for these analytes.

For respiratory rate evaluation, no covariate was included because pre-experiment (baseline) respiratory rate measurements were not available, and the model had week of sampling, time of day (10:00 and 17:00 h), and treatment as fixed effects, with block, experimental unit (cow), and treatment-by-time interaction as random effects, as per the following model:

$$\boldsymbol{Y_{ijkl} = \mu + T_i + H_m + W_l + B_j + V_k(B) + T\ x\ W_{il} + T\ x\ H_{im} + E_{ijklm}}$$

Where: $\boldsymbol{Y}$ = dependent variable; $\boldsymbol{\mu}$ = overall mean; $\boldsymbol{T_i}$ = fixed effect of treatment (i = CON or RPM); $\boldsymbol{H_m}$ = fixed effect time of day (10:00 or 17:00 h); $\boldsymbol{W_l}$ = fixed effect of week (l = 3, 6 or 9); $\boldsymbol{B_j}$ = random effect of block (j = 1–40); $\boldsymbol{V_k(B)}$ = random effect of the experimental unit nested within block (k = 1-80); $\boldsymbol{T\ x\ W_{il}}$ = treatment-by-week interaction; $\boldsymbol{T\ x\ H_{im}}$ = treatment-by-time interaction, and $\boldsymbol{E_{ijklm}}$ = residual error.

Data normality was assessed using the Shapiro-Wilk test ($P < 0.10$). When normality assumptions were not met, variables were analyzed using rank transformation (PROC RANK). Rank-transformed variables included milk composition variables expressed as percentages (milk fat, milk protein, lactose, total solids, and casein), somatic cell count, albumin, HDL cholesterol, ALT, AST, ferric reducing antioxidant power (FRAP), non-esterified fatty acids (NEFA), insulin, cortisol, and respiratory rate. P-values were obtained from models fitted on the rank-transformed data, whereas results are presented in the original units for interpretation.

Covariance structures were tested, and the corrected Akaike Information Criterion (AICc) was used to determine the best covariance structure. Studentized residuals were used to detect and remove outliers (≥ 3 or ≤ -3). The Least-squares means (LSMEANS) statement was used for mean comparisons, with Tukey-Kramer adjustment for significant effects. Significance was declared at $P \leq 0.05$, and trends were noted at $0.05 < P \leq 0.10$.

All outcomes were recorded at the cow level and analyzed using cow-level repeated-measures models; however, because treatments were applied at the pen level (one pen per treatment), pen-level replication was not available.

Results

Environmental conditions

Temperature and relative humidity were recorded inside the compost barn (in-barn microclimate) under the cooling system. During the experimental period, the average ambient temperature was 22.3 ± 1.6 °C, with an average daily maximum of 27.4 ± 2.9 °C and a minimum of 18.8 ± 1.0 °C. Relative humidity averaged 84.7 ± 5.8%, ranging from 41.7 to 100%. The mean temperature-humidity index (THI) was 70.6 ± 2.0, with THI values exceeding 68 and 72 for 68.3% and 32.5% of the total hours, respectively, indicating sustained environmental challenge (Table 2).

Among the three sampling periods, the greatest thermal challenge occurred during weeks 6 and 9, with THI > 72 for 37.7% and 40.1% of the time, respectively. Week 9 also had the highest mean THI (71.6 ± 1.5), as well as the highest average daily temperature (23.1 ± 1.3 °C) and the highest mean maximum temperature (29.5 ± 2.0 °C). In contrast, week 3 presented the mildest conditions, with the lowest mean THI (69.0 ± 2.2), lowest mean temperature (21.1 ± 1.5 °C), and the smallest proportion of hours above both THI thresholds (THI > 68: 56.1%; THI > 72: 19.8%).

Table 2. Mean ambient temperature, relative humidity, and temperature-humidity index (THI) during the monitored sampling weeks (weeks 3, 6, and 9) of the 9-week (63-d) trial conducted in lactating primiparous Holstein cows.

Item	Overall (weeks 3, 6, and 9)	Week 3	Week 6	Week 9
THI[1] > 68, % of time	68.3	56.1	76.5	72.5
THI > 72, % of time	32.5	19.8	37.7	40.1
THI mean	70.6	69.0	71.2	71.6
THI maximum	84.4	84.4	80.1	82.1
THI minimum	61.0	61.0	63.6	64.6
Temperature mean, °C	22.3	21.1	22.7	23.1
Temperature maximum, °C	33.0	31.8	31.8	33.0
Temperature minimum, °C	16.1	16.1	17.6	18.2
Relative humidity mean, %	84.7	88.7	83.3	82.2
Relative humidity maximum, %	100.0	100.0	100.0	100.0
Relative humidity minimum, %	41.7	59.2	43.3	41.7

[1] THI = temperature-humidity index, calculated as THI = (0.8 × T) + [RH × (T – 14.4)] + 46.4, where T = temperature (°C), RH = relative humidity (decimal) [36].
[2] Week = sampling week (evaluation week) as defined in the Methods (weeks 3, 6, and 9 of the trial).
[3] Overall values were calculated from pooled hourly environmental records across sampling weeks 3, 6, and 9.
[4] Air temperature and relative humidity were measured inside the compost barn and reflect the in-barn microclimate under the cooling system (fans and sprinklers).

The daily distribution of THI revealed a clear pattern, with the lowest values occurring between 00:00 and 06:00 h and the highest values between 12:00 and 16:00 h. During this peak period, most THI observations surpassed the threshold of 72, indicating consistent exposure to moderate-to-severe heat challenge in the afternoon hours (Fig 1A).

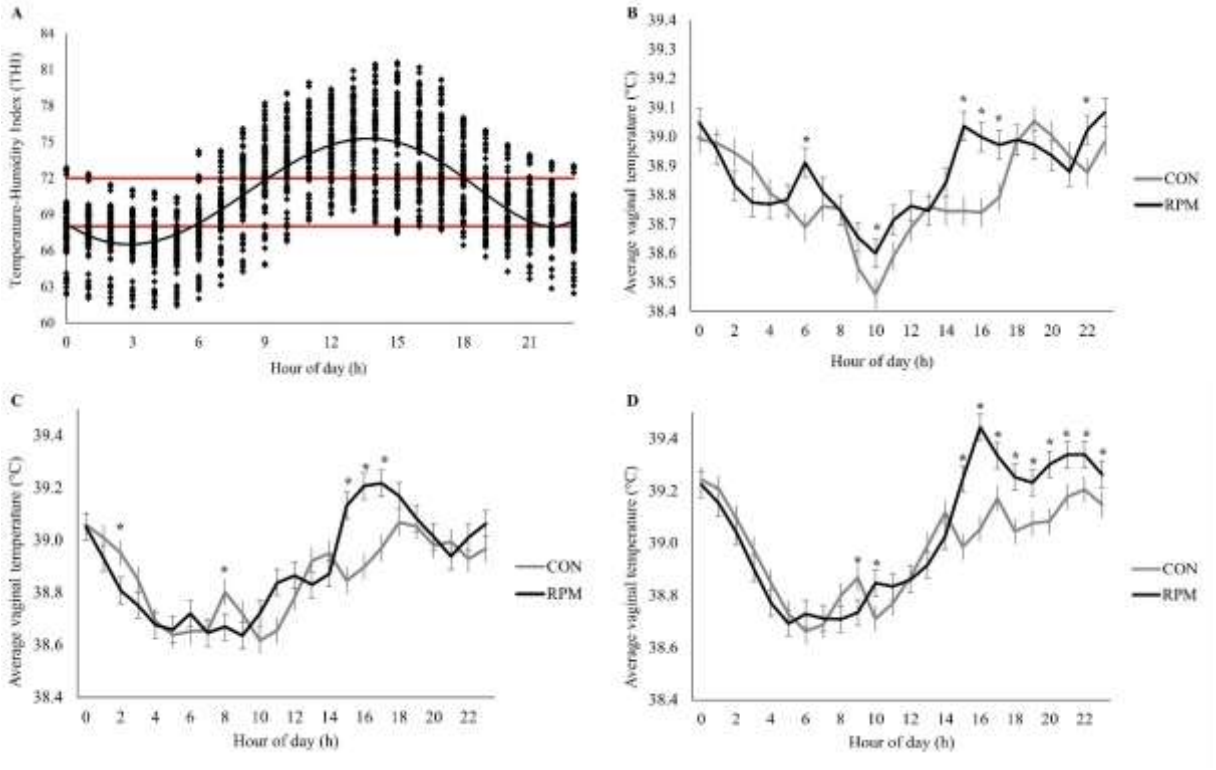


Fig 1. Temperature-humidity index (THI) and vaginal temperature profiles over a 24-h cycle during the sampling weeks (weeks 3, 6, and 9).

(A) Variation of the temperature-humidity index (THI) recorded over a 24-h period. Each point represents an individual hourly measurement, and the solid line represents the smoothed trend across time. Data correspond to the combined observations from the sampling weeks (weeks 3, 6, and 9). Horizontal reference lines indicate THI thresholds of 68 and 72. THI was calculated as $(0.8 \times T) + [RH \times (T - 14.4)] + 46.4$, where T = temperature (°C) and RH = relative humidity expressed as a decimal (Mader et al., 2006).

(B–D) Average vaginal temperature (°C) measured hourly over a 24-h period in lactating primiparous Holstein cows assigned to a control diet (CON; no added rumen-protected methionine) or supplemented with rumen-protected methionine (RPM; 0.75 g/kg diet DM) during sampling week 3 (B), week 6 (C), and week 9 (D) during the Brazilian summer. Bars represent means ± standard error of the mean (SEM). Asterisks (*) indicate time points with treatment differences ($P \leq 0.05$).

Vaginal temperature and respiratory rate

Vaginal temperature and RR are presented in Table 3. No differences were detected between treatments for mean VT, maximum VT, or minimum VT. However, a significant treatment × time (week) × hour interaction was observed for VT ($P < 0.001$), as illustrated in Fig1B-D. In week 3 (Fig 1B), cows supplemented with RPM exhibited higher VT than CON at 06:00, 10:00, 15:00, 16:00, 17:00, and 22:00 h ($P < 0.05$). In week 6 (Fig 1C), differences were detected at 02:00, 08:00, and from 15:00 to 17:00 h, with RPM cows presenting higher VT ($P < 0.05$). In week 9 (Fig 1D), RPM cows showed higher VT at 09:00 and 10:00, and from 15:00 to 23:00 h compared with CON ($P < 0.05$).

Table 3. Respiratory rate (n = 40) and vaginal temperature (n = 15) of primiparous Holstein cows supplemented (RPM) or not (CON) with rumen-protected methionine during summer.

Item	Treatment		SEM	*P*-value			
	CON	RPM		Trt	Week	Trt × Week	Trt × Week × Hour
Respiratory rate at 10:00 h, breaths/min	47.58	51.02	0.90	**0.009**	**< 0.001**	0.405	NA
Respiratory rate at 17:00 h, breaths/min	51.51	57.91	1.16	**< 0.001**	**< 0.001**	**0.024**	NA
Average vaginal temperature, °C	38.88	38.93	0.03	0.293	**< 0.001**	0.435	**< 0.001**
Minimum vaginal temperature, °C	38.75	38.79	0.03	0.302	**< 0.001**	0.638	**< 0.001**
Maximum vaginal temperature, °C	39.01	39.06	0.03	0.341	**< 0.001**	0.103	**< 0.001**

[1]CON = control diet (no added rumen-protected methionine); RPM = rumen-protected methionine–supplemented diet (0.75 g/kg diet DM); SEM = standard error of the mean; Trt = treatment effect; Week = sampling week (weeks 3, 6, and 9); Hour = clock hour for vaginal temperature measurement (hourly values across 24 h); Trt × Week = treatment × sampling week interaction; Trt × Week × Hour = treatment × sampling week × measurement hour interaction. For respiratory rate, measurements were recorded at 10:00 and 17:00 h and the Trt × Week ×

Hour term was not applicable (NA). NA = not applicable (term not included in the model). Boldface P-values indicate statistical significance ($P \leq 0.05$). Measurements were collected in lactating primiparous Holstein cows during the Brazilian summer.

Cows fed the RPM diet showed higher RR at 17:00 h (57.9 vs. 51.5 breaths/min; $P < 0.001$), and a tendency for greater RR at 10:00 h (51.0 vs. 47.6 breaths/min; $P = 0.09$), consistent with the observed interaction between treatment and time at 17:00 h ($P = 0.024$). During respiratory rate measurements (10:00 and 17:00 h), the proportion of cows with RR ≥ 60 breaths/min was higher in the afternoon and increased in weeks 6 and 9. At 17:00 h, RR ≥ 60 breaths/min occurred in 19.2%, 66.2%, and 60.3% of RPM cows and 5.0%, 21.3%, and 42.3% of CON cows in weeks 3, 6, and 9, respectively; at 10:00 h, corresponding proportions were 2.6%, 32.4%, and 39.7% (RPM) and 0.0%, 20.0%, and 30.8% (CON).

Milk production and composition

Milk yield and composition are presented in Table 4. Supplementation with RPM increased yields of milk (44.9 vs 42.9 kg/d, $P < 0.01$), protein (1464 vs 1414 g/d, $P = 0.03$), lactose (2109 vs 2001 g/d, $P = 0.03$) and total solids (5331 vs 5155 g/d, $P = 0.03$). Energy-corrected milk (42.1 vs 40.7 kg/d, $P = 0.06$) and casein yield (1156 vs 1121 g/d, $P = 0.06$) tended to be increased by the RPM treatment. Lactose concentration was reduced in RPM group compared with CON (4.71 vs. 4.76%; $P = 0.01$). Fat yield was not affected by RPM supplementation, but there was an interaction ($P = 0.02$) between treatment and week for fat content (Fig 2). For the RPM treatment, fat content was greater in week 9 compared to week 3, whereas in the control group it did not change significantly across weeks.

Table 4. Milk yield and composition of primiparous Holstein cows supplemented (RPM) or not (CON) with rumen-protected methionine during summer.

Item	Treatment		SEM	*P*-value		
	CON	RPM		Trt	Week	Trt × Week
Milk yield, kg/d	42.9	44.9	0.54	**0.001**	**0.001**	0.302
ECM[2], kg/d	40.7	42.1	0.63	0.063	**< 0.001**	0.177
Milk component yield, g/d						
Fat	1269	1316	38.65	0.363	**< 0.001**	0.133
Protein	1414	1464	17.14	**0.032**	0.319	0.967
Lactose	2001	2109	23.24	**0.034**	**< 0.001**	0.528
Casein	1121	1156	14.61	0.063	0.253	0.950
Total solids	5155	5331	68.81	**0.038**	**< 0.001**	0.381
Milk composition						
Fat, %	2.95	3.02	0.10	0.767	**< 0.001**	**0.020**
Protein, %	3.30	3.27	0.02	0.248	**< 0.001**	0.097
Lactose, %	4.76	4.71	0.01	**0.010**	**0.006**	0.982
Casein, %	2.61	2.58	0.02	0.222	**< 0.001**	0.170
Total solids, %	11.99	11.97	0.10	0.855	**< 0.001**	0.109
MUN[3], mg/dL	11.77	11.46	0.18	0.141	**< 0.001**	0.804
SCC[4] (x 10^3), cells/mL	132.17	178.82	30.23	0.594	0.958	0.378

[1] CON = control diet (no added rumen-protected methionine); RPM = rumen-protected methionine–supplemented diet (0.75 g/kg diet DM); SEM = standard error of the mean; Trt = treatment effect; Week = effect of sampling week (weeks 3, 6, and 9); Trt × Week = treatment × sampling week interaction. Boldface P-values indicate statistical significance ($P \leq 0.05$). Measurements were collected in lactating primiparous Holstein cows during the Brazilian summer.

[2] Energy-corrected milk (ECM) was calculated as ECM (kg/d) = (0.327 × milk yield, kg) + (12.95 × fat yield, kg) + (7.2 × protein yield, kg), according to NASEM (2021).

[3] MUN = milk urea nitrogen.

[4] SCC = somatic cell count.

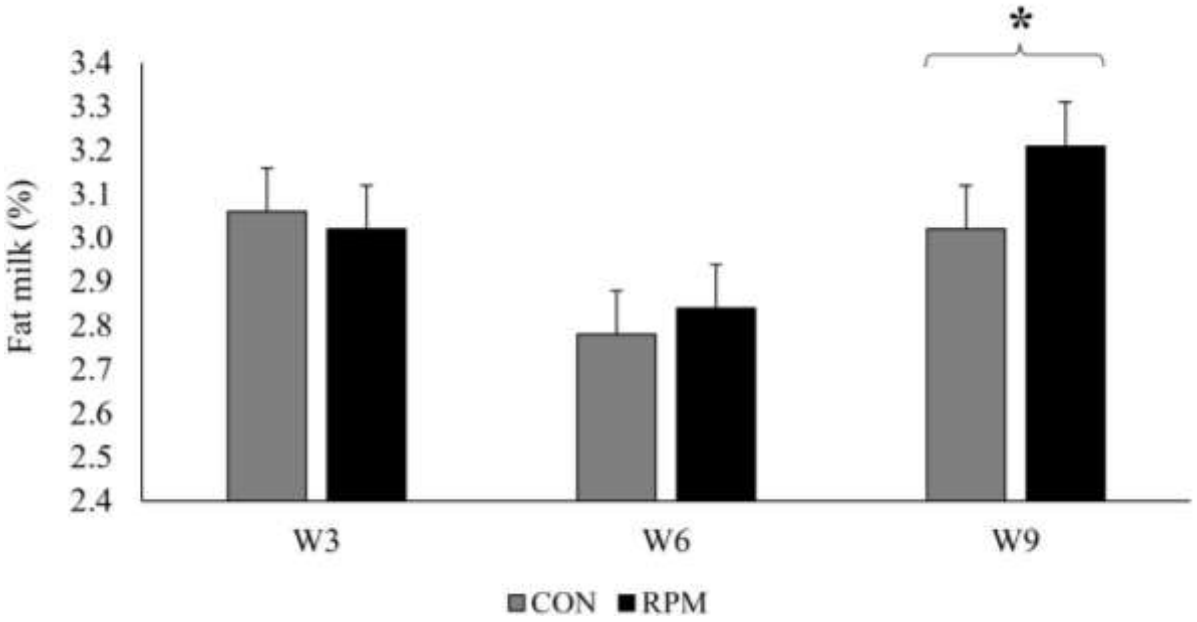


Fig 2. Milk fat concentration (%) during the sampling weeks (weeks 3, 6, and 9) in lactating primiparous Holstein cows assigned to a control diet (CON; no added rumen-protected methionine) or supplemented with rumen-protected methionine (RPM; 0.75 g/kg diet DM) during the Brazilian summer. Bars represent means ± standard error of the mean (SEM). Asterisk (*) above the bracket indicates a difference between treatments within that sampling week ($P \leq 0.05$).

Milk fatty acid composition and yield are in Table 5. Supplementation with RPM did not affect FA profile for C14:0, C16:0, C18 and C18:1. De novo FA yield was not affected, as well as mixed FA and preformed FA.

However, a significant interaction between treatment and week was observed for preformed fatty acids ($P = 0.02$), and a trend was noted for mixed fatty acids ($P = 0.08$). In RPM-supplemented cows, preformed FA concentration increased in week 9 compared to week 3 (1.44 vs. 1.36%), and mixed FA also increased (0.87 vs. 0.79%). No significant temporal variation was observed in the control group.

Table 5. Milk fatty acid composition and yield of primiparous Holstein cows supplemented (RPM) or not (CON) with rumen-protected methionine during summer.

Item	Treatment		SEM	*P*-value		
	CON	RPM		Trt	Week	Trt × Week
Fatty acids composition, g/100 g of milk						
C14:0	0.268	0.282	0.010	0.242	**< 0.001**	0.116
C16:0	0.804	0.848	0.032	0.301	**< 0.001**	**0.049**
C18:0	0.220	0.222	0.009	0.853	**< 0.001**	**0.028**
C18:1	0.706	0.702	0.012	0.871	**< 0.001**	**0.007**
SFA[2]	1.654	1.732	0.069	0.491	**< 0.001**	**0.063**
UFA[3]	1.144	1.144	0.023	0.941	**< 0.001**	**0.024**
MUFA[4]	0.890	0.886	0.019	0.834	**< 0.001**	**0.027**
PUFA[5]	0.116	0.118	0.013	0.520	**< 0.001**	0.301
Short chain	0.142	0.151	0.014	0.397	**< 0.001**	0.130
Middle chain	1.290	1.357	0.047	0.299	**< 0.001**	**0.058**
Long chain	1.005	1.006	0.031	0.973	**< 0.001**	**0.005**
De novo	0.605	0.630	0.033	0.598	**< 0.001**	0.191
Mixed	0.806	0.827	0.036	0.591	**< 0.001**	**0.065**
Preformed	1.357	1.342	0.026	0.656	**< 0.001**	**0.019**
Fatty acids yield, g/day						
C14:0	115.21	122.50	4.39	0.200	**< 0.001**	0.192
C16:0	345.26	367.32	13.32	0.187	**< 0.001**	**0.092**
C18:0	94.78	95.67	3.89	0.864	**< 0.001**	**0.011**
C18:1	306.55	307.16	7.67	0.950	**< 0.001**	**0.001**
SFA[2]	710.92	749.88	27.50	0.297	**< 0.001**	**0.059**
UFA[3]	496.87	501.85	11.02	0.732	**< 0.001**	**0.013**
MUFA[4]	386.85	388.11	8.53	0.843	**< 0.001**	**0.013**
PUFA[5]	50.19	51.94	1.21	0.307	**< 0.001**	0.166
Short chain	59.90	64.81	5.97	0.331	**< 0.001**	0.109
Middle chain	554.38	588.97	19.99	0.156	**< 0.001**	0.106
Long chain	435.96	437.65	13.03	0.897	**< 0.001**	**0.002**
De novo	259.25	271.02	13.81	0.537	**< 0.001**	0.318
Mixed	348.00	357.05	15.32	0.586	0.160	**0.078**
Preformed	589.87	585.86	11.67	0.813	**< 0.001**	**0.019**

[1] CON = control diet (no added rumen-protected methionine); RPM = rumen-protected methionine–supplemented diet (0.75 g/kg diet DM); SEM = standard error of the mean; Trt = treatment effect; Week = effect of sampling week (weeks 3, 6, and 9); Trt × Week = treatment × sampling week interaction. Fatty acids are reported as carbon chain length: number of double bonds (e.g., C16:0). Boldface P-values indicate statistical significance ($P \leq 0.05$). Measurements were collected in lactating primiparous Holstein cows during the Brazilian summer.

[2] SFA = saturated fatty acids.

[3] UFA = unsaturated fatty acids.

[4] MUFA = monounsaturated fatty acids.

[5] PUFA = polyunsaturated fatty acids.

Biochemical, oxidative, immune, and hormonal parameters

Plasma concentrations of biochemical, oxidative stress, inflammatory, immune, and hormonal markers are presented in Table 6. Among the biochemical variables, plasma glucose was significantly lower in RPM compared with CON (39.3 vs. 43.2 mg/dL; $P < 0.001$), whereas other indicators such as albumin, alanine aminotransferase, high-density lipoprotein, and total cholesterol were not affected by treatment. A time effect was observed for plasma urea concentrations ($P = 0.048$), but no treatment effect or treatment × time interaction was detected.

Regarding oxidative stress markers, RPM supplementation enhanced systemic antioxidant status, as evidenced by higher FRAP (32.9 vs. 28.5 μM; $P = 0.006$) and lower plasma MDA concentrations (2.48 vs. 2.78 nmol/mL; $P = 0.007$). No overall treatment effects were observed for GPX or SOD. However, GPX activity was affected by time ($P < 0.001$), and a treatment × time interaction was detected ($P = 0.046$), indicating distinct temporal responses between groups. As shown in Fig 3, GPX concentrations remained stable across weeks in CON cows, whereas RPM cows exhibited a progressive increase, reaching significantly higher values at week 9 compared with week 3 ($P < 0.05$), and also exceeding CON at that final time point.

Inflammatory and immune parameters were unaffected by treatment. Concentrations of haptoglobin and immunoglobulin A did not differ between groups.

Among hormonal variables, RPM-supplemented cows showed higher plasma insulin concentrations (0.52 vs. 0.35 ng/mL; $P < 0.001$), whereas cortisol and non-esterified fatty acids were unaffected.

Table 6. Plasma concentrations of biochemical, oxidative stress, inflammatory, immune, and hormonal parameters of primiparous Holstein cows supplemented (RPM) or not (CON) with rumen-protected methionine during summer.

Item	Treatment		SEM	*P*-value		
	CON	RPM		Trt	Week	Trt × Week
Biochemical parameters						
Albumin, mg/dL	3.40	3.42	0.02	0.363	0.921	0.218
Alanine aminotransferase (ALT), U/L	20.16	21.11	0.54	0.165	0.100	0.137
Aspartate aminotransferase (AST), U/L	111.31	108.00	4.64	0.345	0.115	0.448
Glucose, mg/dL	43.18	39.31	0.84	**< 0.001**	0.102	0.272
High-density lipoprotein (HDL), mg/dL	95.32	95.46	1.40	0.892	**< 0.001**	0.444
Total cholesterol, mg/dL	207.11	211.85	4.51	0.450	0.645	0.368
Urea, mg/dL	35.97	36.54	0.28	0.131	**0.048**	0.530
Oxidative stress biomarkers						
Ferric reducing antioxidant power (FRAP), µM	28.49	32.88	1.16	**0.006**	NA	NA
Glutathione peroxidase (GPX), U/mL	0.30	0.30	0.01	0.645	**< 0.001**	**0.046**
Malondialdehyde (MDA), nmol/mL	2.78	2.48	0.08	**0.007**	**< 0.001**	0.071
Superoxide dismutase (SOD), U/mL	44.28	43.21	2.07	0.712	**0.003**	0.797
Inflammatory and immune markers						
Haptoglobin, mg/dL	20.39	15.76	1.78	0.242	NA	NA
Immunoglobulin A (IgA), mg/dL	99.11	106.67	3.91	0.191	NA	NA
Hormonal and metabolic regulation						
Cortisol, ng/mL	12.27	13.64	0.87	0.315	NA	NA
Insulin, ng/mL	0.35	0.52	0.03	**< 0.001**	NA	NA
Non-esterified fatty acids (NEFA), mmol/L	0.25	0.27	0.01	0.478	NA	NA

[1] CON = control diet (no added rumen-protected methionine); RPM = rumen-protected methionine–supplemented diet (0.75 g/kg diet DM); SEM = standard error of the mean; Trt = treatment effect; Week = effect of sampling week (weeks 3, 6, and 9); Trt × Week = treatment × sampling week interaction. Week and Trt × Week are not applicable for variables measured only once during the experiment (week 9). NA = not applicable. Boldface P-values indicate statistical significance ($P \leq 0.05$). Measurements were collected in lactating primiparous Holstein cows during the Brazilian summer.

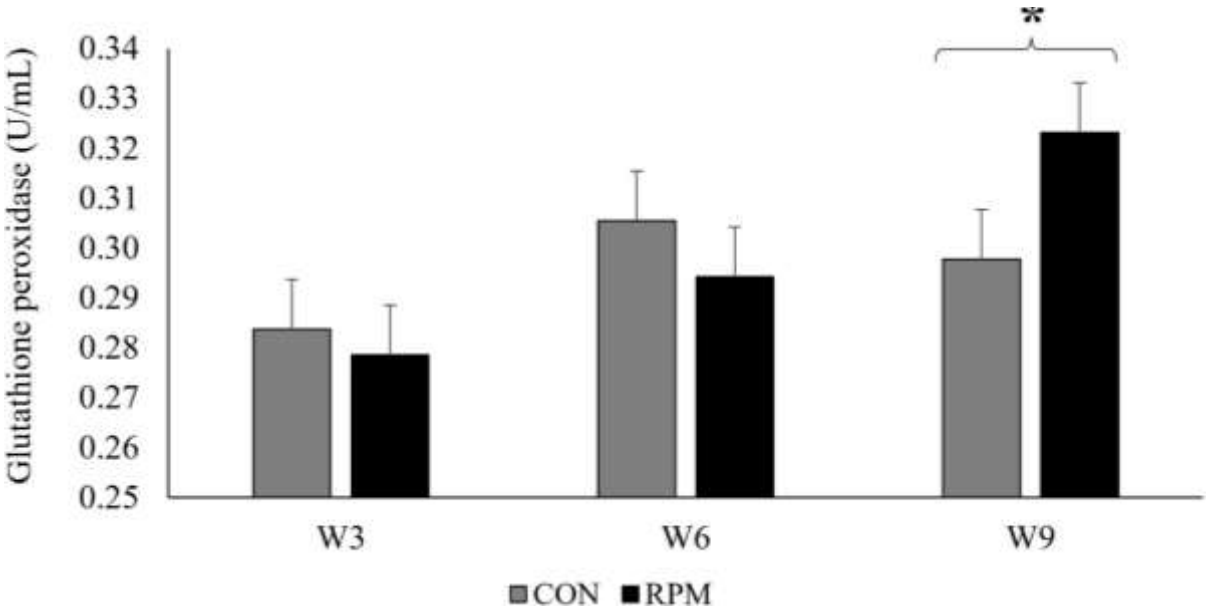


Fig 3. Plasma glutathione peroxidase activity (U/mL) during the sampling weeks (weeks 3, 6, and 9) in lactating primiparous Holstein cows assigned to a control diet (CON; no added rumen-protected methionine) or supplemented with rumen-protected methionine (RPM; 0.75 g/kg diet DM) during the Brazilian summer. Bars represent means ± standard error of the mean (SEM). Asterisk (*) above the bracket indicates a difference between treatments within that sampling week ($P \leq 0.05$).

Discussion

In the present study, supplementation with rumen-protected methionine (RPM) during summer improved milk yield and the daily production of protein, lactose, and total solids in primiparous Holstein cows. RPM also enhanced systemic antioxidant capacity, as evidenced by higher FRAP and lower MDA, and increased plasma insulin concentrations. Although cows receiving RPM exhibited slightly higher respiratory rates and transient increases in vaginal temperature during hotter periods of the day, overall thermal status was not compromised. Collectively, these results indicate that RPM supplementation supported productive performance and metabolic resilience under sustained heat stress conditions.

Thermal comfort in dairy cows is commonly assessed using the THI, which combines ambient temperature and relative humidity. Recent evidence indicates that high genetic merit cows show signs of HS when THI exceeds 68 and experience marked production losses above 72 [41,42]. In the present study, mean THI was 70.6 ± 2.0, with values above 68 and 72 recorded during 68.3% and 32.5% of the monitored hours, respectively, indicating sustained heat load during the monitored sampling weeks and providing the basis for evaluating physiological and metabolic responses to RPM supplementation.

Despite the use of a cooling system, environmental monitoring showed sustained heat load, with THI frequently exceeding 68 and reaching high peak values. Consistent with this pattern, the distribution of respiratory rate observations indicated that a notable proportion of cows exceeded 60 breaths/min at the assessment times, particularly at 17:00 h in weeks 6 and 9. These observations suggest episodic physiological responses compatible with heat challenge during the hottest hours, even under heat-abatement management.

Physiological responses to heat differed between groups. Cows receiving RPM showed higher respiratory rates and transient increases in vaginal temperature during the afternoon hours, particularly in the later weeks. These effects may reflect increased metabolic activity associated with greater milk production rather than impaired thermoregulation, especially given the absence of differences in mean, minimum, or maximum 24-h vaginal temperatures between treatments [22]. The observed rises in vaginal temperature occurred predominantly during periods of higher ambient temperature and may indicate a greater internal heat load, possibly associated with intensified nutrient metabolism to support higher milk production.

In this study, cows supplemented with RPM produced more milk, accompanied by greater daily yields of protein, lactose, and total solids, along with a tendency for higher ECM, even under progressively increasing thermal challenge (mean THI: 69.0, 71.2, and 71.6 during weeks

3, 6, and 9, respectively). This pattern suggests that RPM helped support mammary secretory capacity under summer heat load.

It is important to note that individual DMI was not measured in this study, which limits definitive conclusions regarding the mechanisms underlying the productive responses observed. However, the improvement in the metabolic and oxidative status of RPM-supplemented cows, discussed later, suggests a more favorable physiological condition that may have influenced both nutrient use efficiency and potential voluntary intake. If such an increase in intake occurred, it is plausible that it contributed to part of the observed production response. The diet was formulated to meet the cows' nutritional requirements, and the predicted energy supply was consistent with the observed production levels.

In the present experiment, RPM supplementation increased daily milk protein yield without affecting its concentration and showed a tendency to enhance casein synthesis, suggesting an increased synthetic capacity independent of the dilution effect caused by higher milk volume. Mechanistically, in addition to being a deficient AA for casein synthesis, methionine also acts as a metabolic signal. Through its derivative SAM, it has been linked to activation of the mTOR pathway and stimulates anabolic processes such as protein translation [43,44]. Recent evidence indicates that increasing post-ruminal methionine supply enhances mammary tissue mTOR phosphorylation (p-mTOR), linking it to greater milk protein synthesis in cows exposed to HS [43,18]. Thus, the increase in daily protein yield observed here may reflect both the provision of a deficient AA and the activation of signaling pathways that sustain protein synthesis during HS.

Additionally, HS has been consistently associated with a reduction in milk fat percentage and, in some cases, milk fat yield in dairy cows [25,45-47], although some studies have reported no significant changes [48,49]. Proposed mechanisms include decreased de novo fatty acid synthesis in the mammary gland, associated with reduced DMI and alterations in ruminal

609 fermentation [50-52], as well as potential changes in hepatic triglyceride export [53]. In the
610 present study, the temporal interaction observed for milk fat concentration, with higher values
611 in RPM-supplemented cows in week 9, coincided with an increased proportion of preformed
612 and mixed fatty acids in milk fat, suggesting maintenance of lipogenic capacity even under
613 higher milk production. Although total fat yield remained unchanged, this pattern aligns with
614 previous studies reporting variable effects of RPM on milk fat synthesis depending on basal
615 diet and stage of lactation [54,55]. Methionine may influence milk fat synthesis through
616 multiple pathways. As a lipotropic agent, it participates in the activation of the
617 phosphatidylethanolamine methyltransferase (PEMT) pathway in the liver [56], which converts
618 phosphatidylethanolamine into phosphatidylcholine (PC) using methyl groups donated by
619 SAM. PC is the main phospholipid of very low-density lipoproteins (VLDL), and its increased
620 availability promotes hepatic triglyceride export and enhances the supply of long-chain fatty
621 acids to the mammary gland [21,57-59]. At the mammary level, methionine supplementation
622 has been associated with increased expression of lipogenic genes such as PPARG and FASN,
623 and with reduced abundance of miR-23a, a microRNA that directly represses lipogenic
624 pathways, thereby favoring lipid synthesis [60]. Additionally, methionine acts as a metabolic
625 signal via the mTOR pathway, which regulates key enzymes involved in fatty acid synthesis
626 [61]. The specific impact of rumen-protected methionine supplementation on mammary
627 lipogenesis under HS conditions warrants further investigation.

628 It is also important to highlight that this study was conducted with primiparous cows, which
629 may respond differently to methionine supplementation compared with multiparous cows. In
630 addition to sustaining lactation, primiparous animals must allocate nutrients to ongoing body
631 growth, resulting in altered nutrient partitioning and greater sensitivity to dietary AA adequacy
632 [62,63]. These animals often have lower DMI relative to their requirements [64], a reduced
633 capacity to mobilize body reserves [65] and may respond more positively to increased

metabolizable protein and methionine supply during early and mid-lactation [66,67]. Therefore, the productive responses observed in the present study may reflect a greater nutrient efficiency characteristic of primiparous cows when provided with balanced AA profiles.

Plasma biochemical and hormonal responses provided additional evidence of altered nutrient metabolism. Lower glucose concentrations can be explained by greater lactose production in RPM cows. Moreover, insulin has been demonstrated to be a potent regulator of milk protein synthesis by upregulating genes directly involved in protein translation, casein production, and AA uptake by the mammary gland *ex vivo* [68].

The liver plays a central role in the metabolic adaptation of high-producing cows under HS. Unlike the transition period, HS does not appear to promote pronounced mobilization of non-esterified fatty acids, as adipose tissue lipolysis is suppressed as a physiological strategy to reduce metabolic heat production, thereby exposing the liver to a lower lipid load [4,49]. In this study, serum ALT and AST concentrations remained within physiological ranges, suggesting preserved hepatic function. A temporal increase in HDL concentration was observed, with no treatment or treatment × time effects. HDL mediates reverse cholesterol transport and provides substrate for VLDL formation, which can then be exported to peripheral tissues, including the mammary gland. Although no direct association with treatment was detected, this temporal pattern may have supported lipid availability and contributed to the late increase in milk fat observed in RPM cows at the end of the experiment.

Heat stress exacerbates systemic oxidative stress by promoting an increased generation of reactive oxygen species (ROS) as a consequence of elevated energy demand and intensified mitochondrial activity [69]. This imbalance between ROS production and cellular antioxidant capacity compromises membrane integrity, leading to lipid peroxidation and structural damage. In the present study, a temporal pattern in glutathione peroxidase (GPX) activity was observed, with a significant treatment × time interaction. Although overall GPX means did not differ

between groups, RPM-supplemented cows showed a progressive increase in enzyme activity throughout the weeks, reaching significantly higher values by week 9, whereas levels remained stable in the control group. This pattern paralleled the gradual increase in THI, suggesting that RPM supplementation promoted a more robust antioxidant response to escalating HS conditions. Methionine participates in one-carbon metabolism and, through the transsulfuration pathway, is converted into cysteine, the limiting AA for the synthesis of reduced glutathione, the primary substrate for GPX [70,71]. Thus, RPM supplementation may have enhanced GSH availability and strengthened the antioxidant system dependent on this molecule, increasing the capacity to neutralize lipid and hydrogen peroxides via GPX activity. Reflecting this improved antioxidant efficiency, total antioxidant capacity increased and plasma malondialdehyde, a classical marker of lipid peroxidation [72], decreased in RPM cows. The lower MDA concentration suggests reduced oxidative damage to membranes, while the higher FRAP values indicate greater systemic ability to neutralize ROS. Mechanistic studies in bovine mammary epithelial cells under hyperthermia support this interpretation. Methionine supplementation reduced lipid peroxidation (lower MDA), increased GPX, superoxide dismutase, and catalase activities, and decreased apoptosis [73]. *In vivo*, supplementation with a rumen-protected a methionine–zinc complex enhanced total antioxidant capacity and reduced MDA in cows under prolonged HS, reinforcing the potential of methionine to modulate redox balance, although the effect cannot be entirely separated from zinc in that study [14].

Lactating cows exposed to HS frequently develop a systemic inflammatory response characterized by increased proinflammatory cytokines such as interleukin-1β, interleukin-6, and tumor necrosis factor-α, which stimulate the synthesis of APP such as haptoglobin and lipopolysaccharide-binding protein [7,14,15,19,74]. The severity of this response can be assessed by the rise in circulating haptoglobin, a positive APP, and by the decrease in hepatic albumin synthesis, a negative APP [75]. In the present study, no significant differences between

684 treatments were observed for haptoglobin or albumin. This result may indicate that although
685 HS was sufficient to impact performance and oxidative metabolism, it did not reach the
686 intensity required to induce systemic inflammation capable of markedly altering these APP.
687 The respiration rates reached by the cows during the experiment strengthened this hypothesis,
688 since they were always below 60 movements/min. Alternatively, RPM supplementation may
689 have attenuated the inflammatory response, as suggested by the numeric lower mean
690 haptoglobin concentration in supplemented cows, although not to a magnitude sufficient to
691 achieve statistical significance. It is also important to note that the experimental design, with
692 samples collected every three weeks, may not have captured transient peaks in haptoglobin or
693 rapid declines in albumin concentration that can occur shortly after HS onset.

694 Collectively, these findings support the hypothesis that methionine supplementation
695 enhances lactational performance through coordinated adjustments in nutrient metabolism,
696 antioxidant capacity, and physiological resilience. The consistent productive benefits observed
697 under HS conditions reinforce the potential of RPM to improve production efficiency in hot
698 climates.

699 These findings should be interpreted in light of the study design and on-farm conditions. A
700 key limitation is that dietary treatments were delivered at the pen level (one pen per treatment)
701 and individual daily intake was not measured, which limits pen-level replication and warrants
702 cautious interpretation of treatment effects based on cow-level outcomes. Nonetheless,
703 conducting the trial under commercial summer conditions increases external validity by
704 evaluating RPM responses in a real-world management setting and enabled the inclusion of a
705 larger number of cows than would be feasible with individual feeding.

706

Conclusions

Supplementation with RPM improved milk yield and increased milk protein, lactose, and total solids yields in primiparous Holstein cows during summer. These benefits likely reflect better AA balance and nutrient use. RPM also enhanced antioxidant capacity and increased plasma insulin, suggesting metabolic advantages. Although cows supplemented with RPM exhibited slightly higher vaginal temperatures and respiratory rates during the hottest hours, no detrimental changes or indices of concern in overall thermal status were observed. These findings support the use of methionine to improve performance and metabolic function in primiparous cows under summer heat load, during which THI remained > 68 for extended periods. Further research is warranted to elucidate the tissue-specific mechanisms involved and to evaluate long-term impacts on health and reproductive performance.

Acknowledgments

The authors thank Evonik Operations GmbH (Hanau, Germany) for their collaboration and technical support throughout the study. The authors thank Bioclin (Belo Horizonte, Brazil) for providing reagents, and the INPPAR Research Group at the Federal University of Lavras (Lavras, Brazil) for assistance with data collection and animal management. The authors are also grateful to Fazenda Fazendinha (Três Corações, Brazil) for providing animals and facilities, and to the Animal Physiology Laboratory at the School of Animal Science and Food Engineering, University of São Paulo (FZEA-USP, Pirassununga, Brazil), for conducting part of the laboratory analyses.

References

1. Laporta J, Ferreira FC, Ouellet V, Dado-Senn B, Almeida AK, Dahl GE, et al. Late-

731 gestation heat stress impairs daughter and granddaughter lifetime performance. J Dairy Sci.
732 2020;103:7555–7568. doi:10.3168/jds.2020-18154
733 2. Roth Z. Reproductive physiology and endocrinology responses of cows exposed to
734 environmental heat stress—experiences from the past and lessons for the present.
735 Theriogenology. 2020;155:150–156. doi:10.1016/j.theriogenology.2020.05.040
736 3. IPCC. Climate change 2021: the physical science basis. Contribution of Working Group I
737 to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.
738 Cambridge: Cambridge University Press; 2021 [cited 2024 Oct 15]. Available from:
739 https://www.ipcc.ch/report/ar6/wg1/
740 4. Baumgard LH, Rhoads RP Jr. Effects of heat stress on postabsorptive metabolism and
741 energetics. Annu Rev Anim Biosci. 2013;1:311–337. doi:10.1146/annurev-animal-
742 031412-103644
743 5. Bagath M, Krishnan G, Devaraj C, Rashamol VP, Pragna P, Lees AM, et al. The impact of
744 heat stress on the immune system in dairy cattle: a review. Res Vet Sci. 2019;126:94–102.
745 doi:10.1016/j.rvsc.2019.08.011
746 6. Becker CA, Collier RJ, Stone AE. Invited review: physiological and behavioral effects of
747 heat stress in dairy cows. J Dairy Sci. 2020;103:6751–6770. doi:10.3168/jds.2019-17929
748 7. Ríus AG. Invited review: adaptations of protein and amino acid metabolism to heat stress
749 in dairy cows and other livestock species. Appl Anim Sci. 2019;35:39–48.
750 doi:10.15232/aas.2018-01805
751 8. Horst EA, Mayorga EJ, Baumgard LH. International symposium on ruminant physiology:
752 integrating our understanding of stress physiology. J Dairy Sci. 2025;108:7675–7695.
753 doi:10.3168/jds.2024-25794
754 9. Legrand A, Schütz KE, Tucker CB. Using water to cool cattle: behavioral and
755 physiological changes associated with voluntary use of cow showers. J Dairy Sci.

2011;94:3376–3386. doi:10.3168/jds.2010-3901

10. Armstrong DV. Heat stress interaction with shade and cooling. J Dairy Sci. 1994;77:2044–2050. doi:10.3168/jds.S0022-0302(94)77149-6
11. Gao ST, Guo ZT, Baumgard LH, Ma L, Bu DP. Cooling ameliorates decreased milk protein metrics in heat-stressed lactating Holstein cows. J Dairy Sci. 2021;104:12139–12152. doi:10.3168/jds.2021-20451
12. Knapp DM, Grummer RR. Response of lactating dairy cows to fat supplementation during heat stress. J Dairy Sci. 1991;74:2573–2579. doi:10.3168/jds.S0022-0302(91)78435-X.
13. Williams SRO, Milner TC, Garner JB, Moate PJ, Jacobs JL, Hannah MC, et al. Dietary fat and betaine supplements offered to lactating cows affect dry matter intake, milk production and body temperature responses to an acute heat challenge. Animals (Basel). 2021;11(11):3110. doi:10.3390/ani11113110.
14. Danesh Mesgaran MD, Kargar H, Janssen R, Mesgaran SD, Ghesmati A, Vatankhah AR. Rumen-protected zinc-methionine dietary inclusion alters dairy cow performance, and oxidative and inflammatory status under long-term environmental heat stress. Front Vet Sci. 2022;9:935939. doi:10.3389/fvets.2022.935939
15. Ruiz-González A, Suissi W, Baumgard LH, Martel-Kennes Y, Chouinard PY, Gervais R, et al. Increased dietary vitamin D3 and calcium partially alleviate heat stress symptoms and inflammation in lactating Holstein cows independent of dietary concentrations of vitamin E and selenium. J Dairy Sci. 2023;106:3984–4001. doi:10.3168/jds.2022-22345
16. Daddam JR, Daniel D, Kra G, Pelech I, Portnick Y, Moallem U, et al. Plant polyphenol extract supplementation affects performance, welfare, and the Nrf2-oxidative stress response in adipose tissue of heat-stressed dairy cows. J Dairy Sci. 2023;106:9807–9821. doi:10.3168/jds.2023-23549
17. Abeyta MA, Al-Qaisi M, Horst EA, Mayorga EJ, Rodriguez-Jimenez S, Goetz BM, et al.

781 Effects of dietary antioxidant supplementation on metabolism and inflammatory biomarkers
782 in heat-stressed dairy cows. J Dairy Sci. 2023;106(2):1441–1452. doi:10.3168/jds.2022-
783 22338
784 18. Pate RT, Luchini D, Cant JP, Baumgard LH, Cardoso FC. Immune and metabolic effects
785 of rumen-protected methionine during a heat stress challenge in lactating Holstein cows. J
786 Anim Sci. 2021;99:skab323. doi:10.1093/jas/skab323
787 19. Pate RT, Luchini D, Murphy MR, Cardoso FC. Effects of rumen-protected methionine on
788 lactation performance and physiological variables during a heat stress challenge in lactating
789 Holstein cows. J Dairy Sci. 2020;103:2800–2813. doi:10.3168/jds.2019-17305
790 20. Davidson BD, Zambon AA, Guadagnin AR, Hoppmann A, Larsen GA, Sherlock DN, et al.
791 Rumen-protected methionine supplementation during the transition period under artificially
792 induced heat stress: Effects on cow-calf performance. J Dairy Sci. 2024;107:8654–8669.
793 doi:10.3168/jds.2024-25028
794 21. McFadden JW, Girard CL, Tao S, Zhou Z, Bernard JK, Duplessis M, et al. Symposium
795 review: one-carbon metabolism and methyl donor nutrition in the dairy cow. J Dairy Sci.
796 2020;103:5668–5683. doi:10.3168/jds.2019-17319
797 22. Zhou Z, Vailati-Riboni M, Trevisi E, Drackley JK, Luchini D, Loor JJ. Better postpartal
798 performance in dairy cows supplemented with rumen-protected methionine compared with
799 choline during the periparturient period. J Dairy Sci. 2016;99:8716–8732.
800 doi:10.3168/jds.2015-10525
801 23. Osorio JS, Ji P, Drackley JK, Luchini D, Loor JJ. Supplemental Smartamine M or
802 MetaSmart during the transition period benefits postpartal cow performance and blood
803 neutrophil function. J Dairy Sci. 2013;96:6248–6263. doi:10.3168/jds.2012-5790
804 24. Batistel F, Arroyo JM, Bellingeri A, Wang L, Saremi B, Parys C, et al. Ethyl-cellulose
805 rumen-protected methionine enhances performance during the periparturient period and

806 early lactation in Holstein dairy cows. J Dairy Sci. 2017;100:7455–7467.
807 doi:10.3168/jds.2017-12689
808 25. Ouellet V, Cabral VE, Fadul-Pacheco L, Charbonneau É. The relationship between the
809 number of consecutive days with heat stress and milk production of Holstein dairy cows
810 raised in a humid continental climate. J Dairy Sci. 2019;102:8537–8545.
811 doi:10.3168/jds.2018-16060
812 26. Lovarelli D, Minozzi G, Arazi A, Guarino M, Tiezzi F. Effect of extended heat stress in
813 dairy cows on productive and behavioral traits. Animal. 2024;18:101089.
814 doi:10.1016/j.animal.2024.101089
815 27. Lees AM, Sejian V, Wallage AL, Steel CC, Mader TL, Lees JC, et al. The impact of heat
816 load on cattle. Animals. 2019;9:322. doi:10.3390/ani9060322
817 28. Castro-Montoya J, Corea EE. Heat stress effects in primiparous and multiparous lactating
818 crossbred cows under a warm environment and their responses to a cooling treatment.
819 Anim Prod Sci. 2020;60:2133–2141. doi:10.1071/AN19398
820 29. Kononoff PJ, Hanford KJ. Technical note: estimating statistical power of mixed models
821 used in dairy nutrition experiments. J Dairy Sci. 2006;89:3968–3971.
822 doi:10.3168/jds.S0022-0302(06)72439-0
823 30. NASEM (National Academies of Sciences, Engineering, and Medicine). Nutrient
824 requirements of dairy cattle. 8th rev. ed. Washington, D.C.: National Academies Press;
825 2021.
826 31. AOAC International. Official methods of analysis. 19th ed. Gaithersburg: AOAC
827 International; 2012. Method 942.05, Method 920.39.
828 32. AOAC International. Official methods of analysis. 16th ed. Arlington: AOAC
829 International; 1996. Method 942.05, Method 954.01, Method 973.18.
830 33. Mertens DR. Gravimetric determination of amylase-treated neutral detergent fiber in feeds

831 with refluxing in beakers or crucibles: collaborative study. J AOAC Int. 2002;85:1217–
832 1240. doi:10.1093/jaoac/85.6.1217
833 34. Goering HK, Van Soest PJ. Forage fiber analyses (apparatus, reagents, procedures, and
834 some applications). Agric. Handb. No. 379. Washington, D.C.: U.S. Department of
835 Agriculture; 1970.
836 35. Hall MB. Determination of starch, including maltooligosaccharides, in animal feeds:
837 comparison of methods and a method recommended for AOAC collaborative study. J
838 AOAC Int. 2009;92:42–49. doi:10.1093/jaoac/92.1.42
839 36. Mader TL, Davis MS, Brown-Brandl T. Environmental factors influencing heat stress in
840 feedlot cattle. J Anim Sci. 2006;84:712–719. doi:10.2527/2006.843712x
841 37. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products:
842 malonaldehyde and 4-hydroxynonenal. Methods Enzymol. 1990;186:407–421.
843 doi:10.1016/0076-6879(90)86134-H
844 38. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation
845 of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem.
846 1974;47:469–474. doi:10.1111/j.1432-1033.1974.tb03714.x
847 39. Flohé L, Günzler WA. Assays of glutathione peroxidase. Methods Enzymol.
848 1984;105:114–121. doi:10.1016/S0076-6879(84)05015-1
849 40. Weber K, Osborn M. The reliability of molecular weight determinations by dodecyl
850 sulfate–polyacrylamide gel electrophoresis. J Biol Chem. 1969;244:4406–4412.
851 doi:10.1016/S0021-9258(18)94333-4
852 41. Collier RJ, Hall LW, Rungrueng S, Zimbleman RB. Quantifying heat stress and its impact
853 on metabolism and performance. In: Proceedings of the 24th Annual Southwest Nutrition
854 & Management Conference; 2012 Feb 23-24; Tempe, AZ. p.74–84.
855 42. Tao S, Orellana RM, Weng X, Marins TN, Dahl GE, Bernard JK. Symposium review: the

influences of heat stress on bovine mammary gland function. J Dairy Sci. 2018;101:5642–5654. doi:10.3168/jds.2017-13727

43. Coleman DN, Totakul P, Onjai-uea N, Aboragah A, Jiang Q, Vailati-Riboni M, et al. Rumen-protected methionine during heat stress alters mTOR, insulin signaling, and one-carbon metabolism protein abundance in liver, and whole-blood transsulfuration pathway genes in Holstein cows. J Dairy Sci. 2022;105:7787–7804. doi:10.3168/jds.2021-21379
44. Arriola Apelo SI, Knapp JR, Hanigan MD. Invited review: current representation and future trends of predicting amino acid utilization in the lactating dairy cow. J Dairy Sci. 2014;97:4000–4017. doi:10.3168/jds.2013-7392
45. Gao ST, Guo J, Quan SY, Nan XM, Sanz Fernandez MV, Baumgard LH, et al. The effects of heat stress on protein metabolism in lactating Holstein cows. J Dairy Sci. 2017;100:5040–5049. doi:10.3168/jds.2016-11913
46. Kadzere CT, Murphy MR, Silanikove N, Maltz E. Heat stress in lactating dairy cows: a review. Livest Prod Sci. 2002;77:59–91. doi:10.1016/S0301-6226(01)00330-X
47. Hou Y, Zhang L, Dong RY, Liang MY, Lu Y, Sun XQ, et al. Comparing responses of dairy cows to short-term and long-term heat stress in climate-controlled chambers. J Dairy Sci. 2021;104:2346–2356. doi:10.3168/jds.2020-18946
48. Rhoads ML, Rhoads RP, Van Baale MJ, Collier RJ, Sanders SR, Weber WJ, et al. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. J Dairy Sci. 2009;92:1986–1997. doi:10.3168/jds.2008-1641
49. Wheelock JB, Rhoads RP, Van Baale MJ, Sanders SR, Baumgard LH. Effects of heat stress on energetic metabolism in lactating Holstein cows. J Dairy Sci. 2010;93:644–655. doi:10.3168/jds.2009-2295
50. Bauman DE, Davis CL, Bucholtz HF. Pathways of fatty acid synthesis and reducing

881 equivalent generation in mammary gland of rat, sow, and cow. Arch Biochem Biophys.
882 1970;140(1):237–244. doi:10.1016/0003-9861(70)90065-6
883 51. Liu Z, Ezernieks V, Wang J, Arachchillage NW, Garner JB, Wales WJ, et al. Heat stress
884 in dairy cattle alters lipid composition of milk. Sci Rep. 2017;7:961. doi:10.1038/s41598-
885 017-01120-9
886 52. Urrutia NL, Harvatine KJ. Acetate dose-dependently stimulates milk fat synthesis in
887 lactating dairy cows. J Nutr. 2017;147:763–769. doi:10.3945/jn.116.245001
888 53. Basiricò L, Morera P, Lacetera N, Ronchi B. Down-regulation of hepatic ApoB100
889 expression during hot season in transition dairy cows. Livest Sci. 2011;137:49–57.
890 54. Zanton GI, Bowman GR, Vazquez-Anon M, Rode LM. Meta-analysis of lactation
891 performance in dairy cows receiving supplemental dietary methionine sources or
892 postruminal infusion of methionine. J Dairy Sci. 2014;97:7085–7101.
893 doi:10.3168/jds.2014-8220
894 55. Patton RA. Effect of rumen-protected methionine on feed intake, milk production, true
895 milk protein concentration, and true milk protein yield, and the factors that influence these
896 effects: a meta-analysis. J Dairy Sci. 2010;93:2105–2118. doi:10.3168/jds.2009-2693
897 56. Zhou Z, Garrow TA, Dong X, Luchini DN, Loor JJ. Hepatic activity and transcription of
898 betaine-homocysteine methyltransferase, methionine synthase, and cystathionine synthase
899 in periparturient dairy cows are altered to different extents by supply of methionine and
900 choline. J Nutr. 2017;147:11–19. doi:10.3945/jn.116.240234
901 57. Bionaz M, Loor JJ. Gene networks driving bovine milk fat synthesis. BMC Genomics.
902 2008;9:366. doi:10.1186/1471-2164-9-366
903 58. Zom RLG, Van Baal J, Goselink RMA, Bakker JA, De Veth MJ, Van Vuuren AM. Effect
904 of rumen-protected choline on performance, blood metabolites, and hepatic
905 triacylglycerols of periparturient dairy cattle. J Dairy Sci. 2011;94:4016–4027.

doi:10.3168/jds.2011-4233

59. Myers WA, Rico JE, Davis AN, Fontoura ABP, Dineen MJ, Tate BN, et al. Effects of abomasal infusions of fatty acids and one-carbon donors on hepatic ceramide and phosphatidylcholine in lactating Holstein dairy cows. J Dairy Sci. 2019;102:7087–7101. doi:10.3168/jds.2018-16200
60. Salama AAK, Duque M, Wang L, Shahzad K, Olivera M, Loor JJ. Enhanced supply of methionine or arginine alters mechanistic target of rapamycin signaling proteins, messenger RNA, and microRNA abundance in heat-stressed bovine mammary epithelial cells in vitro. J Dairy Sci. 2019;102:2469–2480. doi:10.3168/jds.2018-15219
61. Li B, Khan MZ, Khan IM, Ullah Q, Cisang ZM, Zhang N, et al. Genetics, environmental stress, and amino acid supplementation affect lactational performance via mTOR signaling pathway in bovine mammary epithelial cells. Front Genet. 2023;14:1195774. doi:10.3389/fgene.2023.1195774
62. Adachi N, Kusuhara T, Nonaka I, Terada F. Effect of close-up dry period protein level on preparturient nitrogen balance and lactating performance of primigravid and multiparous Holstein cows. Asian-Aust J Anim Sci. 2006;19:831–836. doi:10.5713/ajas.2006.831
63. Husnain A, Santos JEP. Meta-analysis of the effects of prepartum dietary protein on performance of dairy cows. J Dairy Sci. 2019;102:9791–9813. doi:10.3168/jds.2018-16043
64. Bell AW. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J Anim Sci. 1995;73(9):2804–2819. doi:10.2527/1995.7392804x
65. Drackley JK, Overton TR, Douglas GN. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J Dairy Sci. 2001;84(E Suppl):E100–E112. doi:10.3168/jds.S0022-0302(01)70204-4
66. Olmos Colmenero JJ, Broderick GA. Effect of dietary crude protein concentration on milk

931 production and nitrogen utilization in lactating dairy cows. J Dairy Sci. 2006;89:1704–
932 1712. doi:10.3168/jds.S0022-0302(06)72238-X
933 67. Lee C, Hristov AN, Cassidy TW, Heyler KS, Lapierre H, Varga GA, et al. Rumen-
934 protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a
935 metabolizable protein-deficient diet. J Dairy Sci. 2012;95:6042–6056.
936 doi:10.3168/jds.2012-5581
937 68. Menzies KK, Lefèvre C, Macmillan KL, Nicholas KR. Insulin regulates milk protein
938 synthesis at multiple levels in the bovine mammary gland. Funct Integr Genomics.
939 2009;9:197–217. doi:10.1007/s10142-008-0103-x
940 69. Burhans WS, Rossiter Burhans CA, Baumgard LH. Invited review: lethal heat stress—the
941 putative pathophysiology of a deadly disorder in dairy cattle. J Dairy Sci. 2022;105:3716–
942 3735. doi:10.3168/jds.2021-21080
943 70. Brosnan JT, Brosnan ME. The sulfur-containing amino acids: an overview. J Nutr.
944 2006;136:1636S–1640S. doi:10.1093/jn/136.6.1636S
945 71. Loor JJ, Lopreiato V, Palombo V, D'Andrea M. Physiological impact of amino acids
946 during heat stress in ruminants. Anim Front. 2023;13:14–24. doi:10.1093/af/vfad052
947 72. Sehirli O, Tozan A, Omurtag GZ, Cetinel S, Contuk G, Gedik N, et al. Protective effect of
948 resveratrol against naphthalene-induced oxidative stress in mice. Ecotoxicol Environ Saf.
949 2008;71:301–308. doi:10.1016/j.ecoenv.2007.08.023
950 73. Han ZY, Mu T, Yang Z. Methionine protects against hyperthermia-induced cell injury in
951 cultured bovine mammary epithelial cells. Cell Stress Chaperones. 2015;20:109–120.
952 doi:10.1007/s12192-014-0530-7
953 74. Fontoura ABP, Javaid VS, Saínz de la Maza-Escolà N, Salandy SL, Fubini E, Grilli J, et
954 al. Heat stress develops with increased total-tract gut permeability, and dietary organic acid
955 and pure botanical supplementation partly restores lactation performance in Holstein dairy

956 cows. J Dairy Sci. 2022;105:7842–7860. doi:10.3168/jds.2022-21820

957 75. Bertoni G, Trevisi E, Han X, Bionaz M. Effects of inflammatory conditions on liver activity
958 in the puerperium period and consequences for performance in dairy cows. J Dairy Sci.
959 2008;91:3300–3310. doi:10.3168/jds.2008-0995

TERCEIRA PARTE

5 CONSIDERAÇÕES FINAIS

Em sistemas tropicais, a produção e a composição do leite variam ao longo do ano e são moduladas por condições ambientais e estratégias de manejo. No primeiro estudo, observou-se que a gordura e a proteína do leite em sistemas de produção brasileiros apresentam evidências de um ritmo circanual consistente, detectável em nível nacional, de rebanho e de vaca individual, e que a inclusão de variáveis econômicas de curto prazo acrescentou pouca explicação adicional aos modelos sazonais. No segundo estudo, conduzido durante o verão sob estresse térmico, a suplementação com metionina protegida em vacas Holandesas primíparas esteve associada a melhora de marcadores do estado antioxidante e a incrementos na produção de leite e de sólidos. Em conjunto, os resultados indicam que a sazonalidade deve ser considerada na interpretação da composição do leite e no planejamento de manejo, e sugerem que intervenções nutricionais, como a metionina protegida, podem contribuir para atenuar perdas de desempenho em condições de calor.

REFERÊNCIAS

AEBI, H. Catalasein vitro. **Methods in Enzymology**, v. 105, p. 121-126, 1984. DOI: 10.1016/s0076-6879(84)05016-3.

AHMED, N.; BERRIDGE, M.V. Transforming oncogenes regulate glucose transport by increasing transporter affinity for glucose: Contrasting effects of oncogenes and heat stress in a murine marrow-derived cell line. **Life Sci.**, v. 63, p.1887-1903, 1998. DOI: 10.1016/s0024-3205(98)00465-2

ALAMER, M. The role of prolactin in thermoregulation and water balance during heat stress in domestic ruminants. **Asian J. Anim. Vet. Adv**., v. 6, n. 12, p. 1153-1169, 2011. DOI: 10.3923/ajava.2011.1153.1169.

AL-QAISI, M.; HORST, E. A.; KVIDERA, S. K.; MAYORGA, E. J.; TIMMS, L. L.; BAUMGARD, L. H. Technical note: Developing a heat stress model in dairy cows using an electric heat blanket. **J. Dairy Sci.**, v.102, p. 684-689, 2019. DOI: 10.3168/jds.2018-15128

ALLEN, M. S.; BRADFORD, B. J.; OBA, M. Board Invited Review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. **J. Anim. Sci**., v. 87, n. 10, p. 3317-3334, 2009. DOI: 10.2527/jas.2009-1779.

ALLEN, J. D.; HALL, L. W.; COLLIER, R. J.; SMITH, J. F. Effect of core body temperature, time of day, and climate conditions on behavioral patterns of lactating dairy cows experiencing mild to moderate heat stress. **J. Dairy Sci.**, v.98, p. 118-127, 2015. DOI: 10.3168/jds.2013-7704

ALVARENGA, F. A. P.; BORGES, I.; PALKOVIČ, L.; RODINA, J.; ODDY, V. H.; DOBOS, R. C. Using a three-axis accelerometer to identify and classify sheep behaviour at pasture. **Applied Animal Behaviour Science**, v. 181, p. 91-99, 2016. DOI: 10.1016/j.applanim.2016.05.026

AMMER, S.; LAMBERTZ, C.; VON SOOSTEN, D.; ZIMMER, K.; MEYER, U.; DÄNICKE, S.; GAULY, M. Impact of diet composition and temperature-humidity index on water and dry matter intake of high-yielding dairy cows. **J. Anim. Physiol. Anim. Nutr. (Berl.)**, v.102, p.103-113, 2018. DOI: 10.1111/jpn.12664

AMAMOU, H.; BECKERS, Y.; MAHOUACHI, M.; HAMMAMI, H. Thermotolerance indicators related to production and physiological responses to heat stress of holstein cows. **J. Therm. Biol**. v. 82, p. 90-98, 2019. DOI: 10.1016/j.jtherbio.2019.03.016

ANEJA, R.; ODOMS, K.; DUNSMORE, K.; SHANLY, T. P; WONG, H. R. Extracellular heat shock protein-70 induces endotoxin tolerance in THP-1 cells. **J. Immunol**., v. 177, p. 7184-7192, 2006. DOI: 10.4049/jimmunol.177.10.7184

AQUILANO, K.; BALDELLI, S.; CIRIOLO, M. R. Glutathione: New roles in redox signaling for an old antioxidant. **Front. Pharmacol.**, v.5, p. 196h, 2014. DOI: 10.3389/fphar.2014.00196

ARCHER, S. C.; COY, F. M.; WAPENAAR, W.; GREEN, M. J. Association of season and herd size with somatic cell count for cows in Irish, English, and Welsh dairy herds. **Vet J.**, v.

196, p. 515-521, 2013. DOI: 10.1016/j.tvjl.2012.12.004

BAGATH, M.; KRISHNAN, G.; DEVARAJ, C.; RASHAMOL, V.P.; PRAGNA, P.; LEES, A.M.; SEJIAN, V. The impact of heat stress on the immune system in dairy cattle: a review. **Res Vet Sci.** v. 126, p. 94-102, 2019. DOI: 10.1016/j.rvsc.2019.08.011

BATISTEL, F.; ARROYO, J. M.; GARCES, C. I. M.; TREVISI, E.; PARYS, C.; BALLOU, M.A.; CARDOSO, F. C.; LOOR, J. J. Ethyl-cellulose rumen-protected methionine alleviates inflammation and oxidative stress and improves neutrophil function during the periparturient period and early lactation in Holstein dairy cows. **J Dairy Sci.** v. 101:, p. 480-490, 2018. DOI:10.3168/jds.2017-13185

BAUCHART, D.; DURAND, D.; GRUFFAT, D.; CHILLIARD, Y. Mechanism of liver steatosis in early lactation cows: Effects of hepatoprotector agents. **Proc. Cornell Nutr. Conf. Feed Manuf.**, Rochester, NY, USA, p. 49-56, 1998.

BAUMAN, D. E.; VERNON, R. G. Effects of Exogenous Bovine Somatotropin on Lactation. **Annu. Rev. Nutr.** v. 13, p. 437-461, 1993. DOI: 10.1146/annurev.nu.13.070193.002253

BAUMAN, D. E.; GRIINARI, J. M. Nutritional regulation of milk fat synthesis. **Annu. Rev. Nutr**., v. 23, p. 203-227, 2003. DOI: 10.1146/annurev.nutr.23.011702.073408.

BAUMGARD, L. H.; WHEELOCK, J. B.; SANDERS, S. R.; MOORE, C. E.; GREEN, H. B.; WALDRON, M. R.; RHOADS, R. P. Postabsorptive carbohydrate adaptations to heat stress and monensin supplementation in lactating Holstein cows. **J Dairy Sci.** v. 94, p. 5620-5633, 2011. DOI: 10.3168/jds.2011-4462

BAUMGARD, L. H.; RHOADS JR., R. P. Effects of heat stress on postabsorptive metabolism and energetics. **Annu. Rev. Anim. Biosci.**, v. 1, p. 311-337, 2013. DOI: 10.1146/annurev-animal-031412-103644

BECKER, C. A.; COLLIER, R.J.; STONE, A.E. Invited review: physiological and behavioral effects of heat stress in dairy cows. **J Dairy Sci.**, v. 103, p. 6751-6770, 2020. DOI: 10.3168/jds.2019-17929

BEEDE, D. K.; COLLIER, R. J. Potential nutritional strategies for intensively managed cattle during thermal stress. **J. Anim. Sci.**, v. 62, p. 543-554, 1986. DOI: 10.2527/jas1986.622543x

BERNABUCCI, U.; CALAMARI, L. Effects of heat stress on bovine milk yield and composition. **Zootec. Nutr. Anim.**, v. 24, p. 247-257, 1998.

BERNABUCCI, U.; RONCHI, B.; LACETERA, N.; NARDONE, A. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. **J Dairy Sci.** v. 85, p. 2173-2179, 2002. DOI: 10.3168/jds.S0022-0302(02)74296-3

BERNABUCCI, U.; LACETERA, N.; BAUMGARD, L. H.; RHOADS, R. P.; RONCHI, B.; NARDONE, A. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. **Animal**, v. 4, p. 1167-1183, 2010. DOI: 10.1017/S175173111000090X

BERNABUCCI, U; BASIRICÒ, L; MORERA, P.; DIPASQUALE, D.; VITALI, A.; CAPPELLI, F. P.; CALAMARI, L. Effect of summer season on milk protein fractions in

Holstein cows. **J Dairy Sci.** v. 98, p. 1815-1827, 2015. DOI:10.3168/jds.2014-8788

BERTONI, G.; TREVISI, E.; HAN, X.; BIONAZ, M. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. **J Dairy Sci.** v. 91, p. 3300-3310, 2008. DOI: 10.3168/jds.2008-0995

BEUTLER E.; DURON, O.; KELLY, B. M. Improved method for the determination of blood glutathione. **J. Lab. Clin. Med.**, v. 61(5), p. 882-888, 1963.

BLUM, J. W.; BRUCKMAIER, R. M.; JANS, F. Rumen-protected methionine fed to dairy cows: Bioavailability and effects on plasma amino acid pattern and plasma metabolite and insulin concentrations. **J. Dairy Sci.**, 82, p. 1991-1998, 1999. DOI: 10.3168/jds.S0022-0302(99)75435-4

BOHMANOVA, J.; MISZTAL, I.; COLE, J. B. Temperature-Humidity Indices as Indicators of Milk Production Losses due to Heat Stress. **J. Dairy Sci**., v. 90, p.1947-1956, 2007. DOI: 10.3168/jds.2006-513

BOOTH, A.; MAGNUSON, A.; FOUTS, J.; FOSTER, M. Adipose tissue, obesity and adipokines: role in cancer promotion. **Horm Mol Biol Clin Invest.**; v. 21: p. 57-74, 2015. DOI: 10.1515/hmbci-2014-0037

BOURDON, L.; BUGUET, A.; CUCHERAT, M.; RADOMSKI, M. W. Use of a spreadsheet program for circadian analysis of biological/physiological data. **Aviat. Space Environ. Med**., v. 66, p. 787-791, 1995.

BRADFORD, B. J.; ALLEN, M. S. Milk fat responses to a change in diet fermentability vary by production level in dairy cattle. **J. Dairy Sci**., v. 87, n. 11, p. 3800-3807, 2004. DOI: 10.3168/jds.S0022-0302(04)73519-5.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Instrução Normativa nº 76, de 26 de novembro de 2018. Regulamentos técnicos de identidade e qualidade do leite cru refrigerado, leite pasteurizado e leite pasteurizado tipo A**. Diário Oficial da União, Brasília, DF, 30 nov. 2018. Disponível em: https://wikisda.agricultura.gov.br/ dipoa_baselegal/in_76-2018_rtiq_leite.pdf. Acesso em: 05 nov. 2025.

BRASIL. Ministério da Agricultura e Pecuária. **Mapa do Leite.** Brasília, DF: MAPA, 2024. Disponível em: https://www.gov.br/agricultura/pt-br/assuntos/producao-animal/mapa-do-leite. Acesso em: 05 nov. 2025.

BRITO, J. R. F. et al. Fatores que influenciam a qualidade do leite. In: **Congresso brasileiro de qualidade do leite,** 10., 2024, Florianópolis. Anais [...]. Florianópolis: CBQL, 2024. p. 107-112.

BUFFINGTON, D. E.; COLLIER, R. J.; CANTON, G. H. Shade management systems to reduce heat stress for dairy cows in hot, humid climates. **Transactions of the ASAE**, v. 26 (6), p. 1798-1802, 1983. DOI: 10.13031/2013.33845

BUSANELLO, M.; ROSSI, R. S.; CASSOLI, L. D.; PANTOJA, J. C. F.; MACHADO, P. F. Estimation of prevalence and incidence of subclinical mastitis in a large population of Brazilian

dairy herds. **J. Dairy Sci.**, v. 100, n. 8, p. 6545-6553, 2017. DOI: 10.3168/jds.2016-12042.

CARABAÑO, M.J.; LOGAR, B.; BORMANN, J.; MINET, J.; VANROBAYS, M.-L.; DÍAZ, C.; TYCHON, B.; GENGLER, N.; HAMMAMI, H. Modeling heat stress under different environmental conditions. **J. Dairy Sci.,** v. 99, p. 3798-3814, 2016. DOI: 10.3168/jds.2015-10212

CARABAÑO, M.J.; RAMÓN, M.; MENÉNDEZ-BUXADERA, A.; MOLINA, A.; DÍAZ, C. Selecting for heat tolerance. **Anim. Front.**, v. 9, p. 62–68, 2019. DOI: 10.1093/af/vfy033

CARVALHO, G. R.; CHAVES, D. O.; ROCHA, D. T. **Pagamento por volume: um desafio para pequenos produtores de leite. Milkpoint,** 2021. Disponível em: https://www.milkpoint.com.br/noticias-emercado/panorama-mercado/diferencial-de-precos - por-volume-um-desafio-para-os-pequenosprodutores-224695/. Acesso em: 05 nov. 2025.

CARVALHO, M. P. **A grande transformação.** Milkpoint, 2021. Disponível em: <https://www.milkpoint.com.br/colunas/marcelo-pereira-de-carvalho/a-grande-transformac ao 227948/>. Acesso em: 05 nov. 2025.

CASTRO-MONTOYA, J.; COREA, E. E. Heat stress effects in primiparous and multiparous lactating crossbred cows under a warm environment and their responses to a cooling treatment. **Animal Production Science**, v. 60, p. 2133-2141, 2020. DOI: 10.1071/AN19398.

CHALFUN, L. T. F. **Produção e composição do leite de diferentes grupamentos genéticos da raça Holandesa**. 2009. Tese (Doutorado em Tecnologia de Alimentos) – Universidade Federal de Lavras, Lavras.

CHANDLER, T. L.; WHITE, H. M. Choline and methionine differentially alter methyl carbon metabolism in bovine neonatal hepatocytes. **PLoS One**, v. 12, e0171080, 2017. DOI: 10.1371/journal.pone.0171080

CHEW, B. P. et al. Variables associated with peripartum traits in dairy cows. IV. Seasonal relationships among temperature, photoperiod, and blood plasma prolactin. **J. Dairy Sci**., v. 62, n. 9, p. 1394-1398, 1979. DOI: 10.3168/jds.S0022-0302(79)83435-9.

CLARK, K. L.; RABELO, L. R.; LEE, C. Use of the Se-yeast method to estimate absolute bioavailability of rumen protected methionine. In: Abstracts of the 2023 American Dairy Science Association Annual Meeting. **J. Dairy Sci.** Vol. 106, Suppl. 1, p. 302, 2023. Disponível em: < https://www.adsa.org/Portals/0/SiteContent/Docs /Meetings/2023ADSA/Abstracts_BOOK_2023.pdf>. Acesso em: 15/08/23.

COLLIER, R.J.; BEEDE, D.K.; THATCHER, W.W.; ISRAEL, L.A.; WILCOX, C.J. Influences of Environment and Its Modification on Dairy Animal Health and Production. **J. Dairy Sci.**, v. 65, p. 2213-2227, 1982. DOI: 10.3168/jds.S0022-0302(82)82484-3

COLLIER, R. J.; COLLIER, J. L.; RHOADS, R. P.; BAUMGARD, L. H. Invited Review: Genes involved in the bovine heat stress response. **J. Dairy Sci.**, v. 91, p. 445-454, 2008. DOI: 10.3168/jds.2007-0540

COLLIER, R.J.; BAUMGARD, L.H.; ZIMBELMAN, R.B.; XIAO, Y. Heat stress: Physiology of acclimation and adaptation. **Anim. Front.**, v. 9, p. 12-19, 2019. DOI: 10.1093/af/vfy031
COOK, N. B.; MENTINK, R. L.; BENNETT, T. B.; BURGI, K. The effect of heat stress and lameness on time budgets of lactating dairy cows. **J. Dairy Sci.**, v. 90, p. 1674-1682, 2007. DOI: 10.3168/jds.2006-634

COWLEY, F. C.; BARBER, D.G.; HOULIHAN, A.V.; POPPI, D.P. Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. **J Dairy Sci.**, v. 98, p. 2356-2368, 2015. DOI: 10.3168/jds.2014-8442

COSTA, A.; LOPEZ-VILLALOBOS, N.; SNEDDON, N. W.; SHALLOO, L.; FRANZOI, M.; DE MARCHI, M.; PENASA, M. Invited review: Milk lactose—Current status and future challenges in dairy cattle. J. Dairy Sci., v. 102, n. 7, p. 5883-5898, 2019. DOI: 10.3168/jds.2018-15955..

COUNCIL ON DAIRY CATTLE BREEDING (CDCB). Genetic and phenotypic trends: U.S. dairy cattle. Disponível em: https://queries.uscdcb.com/eval/summary/trend.cfm?R_Menu=HO#StartBod. Acesso em: 05 nov. 2025.

DAHL, G. E.; BUCHANAN, B. A.; TUCKER, H. A. Photoperiodic effects on dairy cattle: a review. **J. Dairy Sci**., v. 83, n. 4, p. 885-893, 2000. DOI: 10.3168/jds.S0022-0302(00)74952-6.

DAS, R.; SAILO, L.; VERMA, N.; BHARTI, P.; SAIKIA, J.; IMTIWATI; KUMAR, R. Impact of heat stress on health and performance of dairy animals: A review. **Veterinary World**, v. 9, n. 3, p. 260-268, 2016. DOI: 10.14202/vetworld.2016.260-268

DEPETERS, E. J.; CANT, J. P. Nutritional factors influencing the nitrogen composition of bovine milk: a review. **J. Dairy Sci**., v. 75, n. 8, p. 2043-2070, 1992. DOI: 10.3168/jds.S0022-0302(92)77964-8.

DOKLADNY, K.; MOSELEY, P.L.; MA, T.Y. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. **Am. J. Physiol. Liver Physiol.**, v. 290, p.204-212, 2006. DOI: 10.1152/ajpgi.00401.2005

DONG, X.; ZHOU, Z.; SAREMI, B.; HELMBRECHT, A.; WANG, Z.; LOOR, J. J. Varying the ratio of Lys:Met while maintaining the ratios of Thr:Phe, Lys:Thr, Lys:His, and Lys:Val alters mammary cellular metabolites, mammalian target of rapamycin signaling, and gene transcription. **J Dairy Sci**. v.101, p.1708-1718, 2018. DOI: 10.3168/jds.2017-13351

DOSKA, M. C. et al. Sources of variation in milk urea nitrogen in Paraná dairy cows. **Rev. Bras. Zootec**., v. 41, n. 3, p. 692-697, 2012.

DROVERS CATTLE NETW. 2011. Heat wave kills as many as 4,000 cattle last week in Iowa. http://www.cattlenetwork.com/cattle-resources/hot-topics/Heat-wave-kills-as-many-as-4000-cattle-last-week-in-Iowa-126763608.html

DU, X.; SHEN, T.; WANG, H.; QIN, X.; XING, D.; YE, Q.; SHI, Z.; FANG, Z.; ZHU, Y.; YANG, Y.; PENG, Z.; ZHAO, C.; LV, B.; LI, X.; LIU, G.; LI, X. Adaptations of hepatic lipid metabolism and mitochondria in dairy cows with mild fatty liver. **J. Dairy Sci.**, v. 101, p. 9544-

9558, 2018. DOI: 10.3168/jds.2018-14546

ELVINGER, F.; NATZKE, R. P.; HANSEN, P. J. Interactions of Heat Stress and Bovine Somatotropin Affecting Physiology and Immunology of Lactating Cows. **J. Dairy Sci.**, v. 75, p. 449-462, 1992. DOI: 10.3168/jds.S0022-0302(92)77781-9

EMBRAPA GADO DE LEITE. Anuário Leite 2025. 8. ed. Juiz de Fora: Embrapa Gado de Leite; Texto Comunicação, 2025. Disponível em: https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/1176413/1/Anuario-Leite-2025.pdf. Acesso em: 05 nov. 2025.

ESTERBAUER, H.; CHEESEMAN, K. H. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. **Methods in Enzymology, v.** 186, p. 407-421, 1990. DOI: 10.1016/0076-6879(90)86134-h

FLESHNER, M.; JOHNSON, J. D. Endogenous extracellular heat shock protein 72: Releasing signal(s) and function. **Int. J. Hyperthermia**, v. 21, p. 457-471, 2005. DOI: 10.1080/02656730500088211

FLOHÉ, L.; GÜNZLER, W. A. Assays of glutathione peroxidase. **Methods in Enzymology**, v. 205, p. 114-121, 1984. DOI: 10.1016/S0076-6879(84)05015-1

FONTOURA, A. B. P; JAVAID, A.; SÁINZ DE LA MAZA-ESCOLÀ, V.; SALANDY, N. S.; FUBINI, S. L.; GRILLI, E.; MCFADDEN, J. W. Heat stress develops with increased total-tract gut permeability, and dietary organic acid and pure botanical supplementation partly restores lactation performance in Holstein dairy cows. **J Dairy Sci,** v. 105, p. 7842–7860, 2022. DOI: 10.3168/jds.2022-21820

FUQUAY, J. Heat stress as it affects animal production. **J. Anim. Sci.**, v. 52, p. 164–174, 1981. DOI: 10.2527/jas1981.521164x

GAO, S.; GUO, J.; QUAN, S.; NAN, X.; FERNANDEZ, M. S.; BAUMGARD, L.; BU, D. The effects of heat stress on protein metabolism in lactating Holstein cows. **J. Dairy Sci.**, v. 100, p. 5040-5049, 2017. DOI: 10.3168/jds.2016-11913

GAO, S. T.; GUO, Z. T.; BAUMGARD, L. H.; MA, L.; BU, D. P. Cooling ameliorates decreased milk protein metrics in heat-stressed lactating Holstein cows. **J. Dairy Sci**., v. 104, n. 11, p. 12139-12152, 2021. DOI: 10.3168/jds.2021-20451.

GAUGHAN, J. B. Basic Principles Involved in Adaption of Livestock to Climate Change. *In*: Environmental Stress and Amelioration in Livestock Production. 1st ed. Berlin/Heidelberg, Germany: Springer, 2012; chap. 10, p. 245-261. DOI: 10.1007/978-3-642-29205-7_10

GIBSON, J. P. Economic weights and index selection of milk production traits when multiple production quotas apply. **Anim. Sci.**, v. 49, n. 2, p. 171-181, 1989. DOI: 10.1017/S0003356100032293

GONZALEZ-RIVAS, P. A.; CHAUHAN, S. S.; HA, M.; FEGAN, N.; DUNSHEA, FR.; WARNER, R. D. Effects of heat stress on animal physiology, metabolism, and meat quality: a review. **Meat Sci.** v. 162, 108025, 2020. DOI: 10.1016/j.meatsci.2019.108025

GUO, J.; GAO, S.; QUAN, S.; ZHANG, Y.; BU, D.; WANG, J. Blood amino acids profile responding to heat stress in dairy cows. **Asian-Australas. J. Anim. Sci.**, v. 31, p. 47-53, 2018. DOI: 10.5713/ajas.16.0428

GUO, Z.; GAO, S.; OUYANG, J.; MA, L.; BU, D. Impacts of heat stress-induced oxidative stress on the milk protein biosynthesis of dairy cows. **Animals**. v. 11, 726, 2021. DOI: 10.3390/ani11030726

HAGIYA, K.; HAYASAKA, K.; YAMAZAKI, T.; SHIRAI, T.; OSAWA, T.; TERAWAKI, Y.; NAGAMINE, Y.; MASUDA, Y.; SUZUKI, M. Effects of heat stress on production, somatic cell score and conception rate in Holsteins. **Anim. Sci. J**., v. 88, p. 3-10, 2017. DOI: 10.1111/asj.12617

HALL, D. M.; BUETTNER, G. R.; OBERLEY, L. W.; XU, L.; MATTHES, R. D.; GISOLFI, C. V. Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. **Am. J. Physiol. Heart Circ. Physiol.**, v. 280, p. 509-521, 2001. DOI: 10.1152/ajpheart.2001.280.2.H509

HALL, M. B. Determination of starch, including maltooligosaccharides, in animal feeds: comparison of methods and a method recommended for AOAC collaborative study. **J AOAC Int**., v. 92(1), p. 42-49, 2008. DOI: 10.1093/jaoac/92.1.42

HAZLERIGG, D.; LOMET, D.; LINCOLN, G.; DARDENTE, H. Neuroendocrine correlates of the critical day length response in the Soay sheep. **J. Neuroendocrinol**., v. 30, n. 9, e12631, 2018. DOI: 10.1111/jne.12631.

HEINICKE, J.; HOFFMANN, G.; AMMON, C.; AMON, B.; AMON, T. Effects of the daily heat load duration exceeding determined heat load thresholds on activity traits of lactating dairy cows. **J. Therm. Biol.**, v. 77, p. 67-74, 2018. DOI: 10.1016/j.jtherbio.2018.08.012

HERBUT, P.; ANGRECKA, S. Relationship between THI level and dairy cows' behaviour during summer period. **Ital. J. Anim. Sci.**, v. 17, p.226-233, 2018. DOI: 10.1080/1828051X.2017.1333892

HILL, D.L.; WALL, E. Dairy cattle in a temperate climate: The effects of weather on milk yield and composition depend on management. **Animal**, v. 9, p. 138-149, 2015. DOI: 10.1017/S1751731114002456

HORST, E. A.; KVIDERA, S. K.; BAUMGARD, L. H. Invited review: the influence of immune activation on transition cow health and performance - A critical evaluation of traditional dogmas. **J Dairy Sci.**, v. 104, p. 8380-8410, 2021. DOI: 10.3168/jds.2021-20330

IBGE. Valor da produção da pecuária e da aquicultura chega a R$ 132,8 bilhões em 2024… Agência de Notícias IBGE, 2025. Disponível em: https://agenciadenoticias.ibge.gov.br/. Acesso em: 05 nov. 2025.

IPEMA, A. H.; GOENSE, D.; HOGEWERF, P .H.; HOUWERS, H. W. J.; VAN ROEST, H. Pilot study to monitor body temperature of dairy cows with a rumen bolus. **Comput. Electron. Agric.**, v. 64, p. 49-52, 2008. DOI: 10.1016/j.compag.2008.05.009

JENKINS, T. C.; HARVATINE, K. J. Lipid feeding and milk fat depression. **Vet. Clin. North Am. Food Anim. Pract.**, v. 30, n. 3, p. 623-642, 2014. DOI: 10.1016/j.cvfa.2014.07.006.
JOHNSON, J. D.; CAMPISI, J.; SHARKEY, C. M.; KENNEDY, S. L.; NICKERSON, M.; FLESHNER, M. Adrenergic receptors mediate stress-induced elevations in extracellular Hsp72. **J. Appl. Physiol.**, v. 99, p. 1789-1795, 2005. DOI: 10.1152/japplphysiol.00390.2005

KADZERE, C. T.; MURPHY, M. R.; SILANIKOVE, N.; MALTZ, E. Heat stress in lactating dairy cows: A review. **Livest. Prod. Sci.**, v. 77, p. 59-91, 2002. DOI: 10.1016/S0301-6226(01)00330-X

KLEIN, B. G. **Cunningham tratado de fisiologia veterinária**. 5. ed., Rio de Janeiro: Elsevier, 2014

KOUKKARI, W. L.; SOTHERN, R. B. **G**eneral features of rhythms: terminology and characteristics. In: KOUKKARI, W. L.; SOTHERN, R. B. **Introducing biological rhythms.** New York: Springer, 2006a. p. 19-65.

KOUKKARI, W. L.; SOTHERN, R. B. **Introducing biological rhythms**. New York: Springer, 2006b.

KRISTENSEN, T. N.; LOVENDAHL, P.; BERG, P.; LOESCHCKE, V. Hsp 72 is present in plasma from Holstein-Friesian dairy cattle, and the concentration level is repeatable across days and age classes. **Cell Stress Chaperones**, v. 9 , p. 143-149, 2004. DOI: 10.1379/CSC-17.1

LABEN, R. C. Factors responsible for variation in milk composition. **J Dairy Sci**, v. 46, n. 11, p. 1293-1301, 1963. DOI: 10.3168/jds.S0022-0302(63)89264-4

LAMBERT, G. P.; GISOLFI, C. V.; BERG, D. J.; MOSELEY, P. L.; OBERLEY, L. W.; KREGEL, K. C. Selected Contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. **J. Appl. Physiol.**, v. 92, p. 1750-1761, 2002. DOI: 10.1152/japplphysiol.00787.2001

LANKS, K. W. Modulators of the eukaryotic heat shock response. **Exp. Cell Res.**, v. 165 , p. 1-10, 1986. DOI: 10.1016/0014-4827(86)90528-8

LEE, W. C.; WEN, H. C.; CHANG, C. P.; CHEN, M. Y.; LIN, M. T. Heat shock protein 72 overexpression protects against hyperthermia, circulatory shock and cerebral ischemia during heat stroke. **J. Appl. Physiol.**, v. 100 , p. 2073-2082, 2006. DOI: 10.1152/japplphysiol.01433.2005

LEE, Y.; BOK, J.D.; LEE, H.J.; LEE, H.G.; KIM, D.; LEE, I.; KANG, S.K.; CHOI, Y.J. Body Temperature Monitoring Using Subcutaneously Implanted Thermo-loggers from Holstein Steers. **Asian-Australas. J. Anim. Sci.**, v. 29, p. 299-306, 2016, DOI: 10.5713/ajas.15.0353

LEGRAND, A.; SCHÜTZ, K. E.; TUCKER, C. B. Using water to cool cattle: Behavioral and physiological changes associated with voluntary use of cow showers. **J. Dairy Sci.**, v. 94, p. 3376-3386, 2011. DOI: 10.3168/jds.2010-3901

LEMERLE, C.; GODDARD, M. E. Assessment of heat stress in dairy cattle in Papua New Guinea. **Trop. Anim. Health Prod.**, v. 18, p. 232-242, 1986. DOI: 10.1007/BF02359540

LI, S.; HOSSEINI, A.; DANES, M.; JACOMETO, C.; LIU, J.; LOOR, J. J. Essential amino acid ratios and mTOR affect lipogenic gene networks and miRNA expression in bovine mammary epithelial cells. **J Anim Sci Biotechnol.** v. 7, p. 44, 2016. DOI: 10.1186/s40104-016-0104-x

LINCOLN, G. A.; ANDERSSON, H.; LOUDON, A. Clock genes in calendar cells as the basis of annual timekeeping in mammals—a unifying hypothesis. **J. Endocrinol**., v. 179, n. 1, p. 1-13, 2003. DOI: 10.1677/joe.0.1790001.

LINDQUIST, S. The heat-shock response. **Annu. Rev. Biochem.**, v. 55, p. 1151-1191, 1986. DOI: 10.1146/annurev.bi.55.070186.005443

LIU, Z.; EZERNIEKS, V.; WANG, J.; ARACHCHILLAGE, N. W.; GARNER, J. B.; WALES, W. J.; COCKS, B. G.; ROCHFORT, S. Heat stress in dairy cattle alters lipid composition of milk. **Sci. Rep.**, v. 7, p. 961, 2017. DOI: 10.1038/s41598-017-01120-9

LOCK, A.; OVERTON, T.; HARVATINE, K.; GIESY, J.; BAUMAN, D. Milk fat depression: impact of dietary components and their interaction during rumen fermentation. P**roc. Cornell Nutr**. Conf., p. 75-85, 2006.

LOOR, J. J.; BIONAZ, M.; DRACKLEY, J. K. Systems physiology in dairy cattle: Nutritional genomics and beyond. **Annu. Rev. Anim. Biosci.**, v. 1, p. 365-392, 2013. DOI: 10.1146/annurev-animal-031412-103728

LOOR, J. J.; BERTONI, G.; HOSSEINI, A.; ROCHE, J.; TREVISI, E. Functional welfare - Using biochemical and molecular technologies to understand better the welfare state of peripartal dairy cattle. **Anim. Prod. Sci.**, v. 53, p. 931-953, 2013. DOI:10.1071/AN12344

LOPREIATO, V.; VAILATI-RIBONI, M.; BELLINGERI, A.; KHAN, I.; FARINA, G.; PARYS, C., LOOR, J. J. Inflammation and oxidative stress transcription profiles due to in vitro supply of methionine with or without choline in unstimulated blood polymorphonuclear leukocytes from lactating Holstein cows. **J Dairy Sci.**, v. 102, p. 10395-10410, 2019. DOI: 10.3168/jds.2019-16413

MACHADO, P. F.; PEREIRA, A. R.; SARRÍES, G. A. Composição do leite de tanques de rebanhos brasileiros distribuídos segundo sua contagem de células somáticas. **Rev. Bras. Zootec**., v. 29, n. 6, p. 1883-1886, 2000. DOI: 10.1590/S1516-35982000000600038.

MARKLUND, S.; MARKLUND, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. **European Journal of Biochemistry**, v. 47, p. 469-474, 1974. DOI: 10.1111/j.1432-1033.1974.tb03714.x

MARTINOV, M. V.; VITVITSKY, V.; BANERJEE, R.; ATAULLAKHANOV, F. The logic of the hepatic methionine metabolic cycle. **Biochim. Biophys. Acta**, v. 1804, p. 89-96, 2010. DOI: 10.1016/j.bbapap.2009.10.004

MAULFAIR, D. D.; MCINTYRE, K. K.; HEINRICHS, A. J. Subacute ruminal acidosis and total mixed ration preference in lactating dairy cows. **J. Dairy Sci**., v. 96, n. 10, p. 6610-6620, 2013. DOI: 10.3168/jds.2013-6771.

MAVANGIRA, V.; SORDILLO, L. M. Role of lipid mediators in the regulation of oxidative stress and inflammatory responses in dairy cattle. **Res. Vet. Sci.**, v. 116 , p. 4-14, 2018. DOI: 10.1016/j.rvsc.2017.08.002

MAVROMMATIS, A.; MITSIOPOULOU, C.; CHRISTODOULOU, C.; KARIAMPA, P.; SIMONI, M.; RIGHI, F.; TSIPLAKOU, E. Effects of supplementing rumen-protected methionine and lysine on milk performance and oxidative status of dairy ewes. **Antioxidants**. v. 10, p. 654, 2021. DOI: 10.3390/antiox10050654

McGOWN, C.; BIRERDINC, A.; YOUNOSSI, Z. M. Adipose tissue as an endocrine organ. **Clin. Liver Dis.**, v. 18, p. 41-58, 2014. DOI: 10.1016/j.cld.2013.09.012

McGUIRE, M. A.; BEEDE, D. K.; DELORENZO, M. A.; WILCOX, C. J.; HUNTINGTON, G. B.; REYNOLDS, C. K.; COLLIER, R. J.. Effects of thermal stress and level of feed intake on portal plasma flow and net fluxes of metabolites in lactating Holstein cows. **J. Anim. Sci.**, v. 67, p. 1050-1060, 1989. DOI: 10.2527/jas1989.6741050x

McGUIRE, M. A.; BEEDE, D. K.; COLLIER, R. J.; BUONOMO, F. C.; DELORENZO, M. A.; WILCOX, C. J.; HUNTINGTON, G. B.; REYNOLDS, C. K. Effects of acute thermal stress and amount of feed intake on concentrations of somatotropin, insulin-like growth factor (IGF)-I and IGF-II, and thyroid hormones in plasma of lactating Holstein cows. **J. Anim. Sci.**, v. 69, p. 2050-2056, 1991. DOI: 10.2527/1991.6952050x

MEIRELLES, C. P. et al. Importância do controle leiteiro na avaliação da composição do leite de vacas jersey no estado do Rio Grande do Sul, Brasil. **Revista de Educação Continuada em Medicina Veterinária e Zootecnia** do CRMV-SP, v. 14, n. 3, p. 79-79, 2016. Disponível em: https://www.revistamvez-crmvsp.com.br/index.php/recmvz/article/view/34923. Acesso em: 15 nov. 2025.

MERTENS, D. R. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. **Journal of AOAC International**, v.85, p.1217-1240, 2002.

MÉTAYER, S.; SEILIEZ, I.; COLLIN, A.; DUCHÊNE, S.; MERCIER, Y.; GERAERT, P-A, TESSERAUD S. Mechanisms through which sulfur amino acids control protein metabolism and oxidative status. **J Nutr Biochem.** v. 19, p. 207-215. DOI: 10.1016/j.jnutbio.2007.05.006

MESGARAN, M. D.; KARGAR, H.; JANSSEN, R.; MESGARAN, S. D.; GHESMATI, A.; VATANKHAN, A. Rumen-protected zinc–methionine dietary inclusion alters dairy cow performances, and oxidative and inflammatory status under long-term environmental heat stress. **Front Vet Sci.,** v. 9, p. 935939, 2022. DOI: 10.3389/fvets.2022.935939

MILLER-CUSHON, E. K.; DEVRIES, T. J. Feed sorting in dairy cattle: Causes, consequences, and management. **J. Dairy Sci**., v. 100, n. 5, p. 4172-4183, 2017.

MIZZEN, L. A.; WELCH, W. J. Characterization of the thermotolerant cell. I. Effects on protein synthesis activity and the regulation of heat-shock protein 70 expression. **J. Cell Biol.**, v. 106, p. 1105–1116, 1988. DOI: 10.1083/jcb.106.4.1105

MOHAMMED, M. E.; JOHNSON, H. D. Effect of growth hormone on milk yields and related physiological functions of Holstein cows exposed to heat stress. **J. Dairy Sci**., v. 68, n. 5, p. 1123-1133, 1985. DOI: 10.3168/jds.S0022-0302(85)80938-3.

MORAES, L. R.; DELICATO, M. E. A.; DA SILVA CRUZ, A.; DA SILVA, H. T. F. N. P.; DE VASCONCELOS ALVES, C. V. B.; CAMPOS D. B.; SARAIVA, E.P.; PERAZZO DA COSTA F.; GUERRA, R. R. Methionine supplementing effects on intestine, liver and uterus morphology, and on positivity and expression of Calbindin-D28k and TRPV6 epithelial calcium carriers in laying quail in thermoneutral conditions and under thermal stress. **PLoS ONE.** v. 16, e0245615, 2021. DOI: 10.1371/journal.pone.0245615

MURRI, M.; INSENSER, M.; LUQUE, M.; TINAHONES, F. J.; ESCOBAR-MORREALE, H. F. Proteomic analysis of adipose tissue: Informing diabetes research. **Expert Rev. Proteomics**, v. 11, p. 491-502, 2014. DOI: 10.1586/14789450.2014.903158

NAN, X.; BU, D.; LI, X.; WANG, J.; WEI, H.; HU, H.; ZHOU, L.; LOOR, J. J. Ratio of lysine to methionine alters expression of genes involved in milk protein transcription and translation and mTOR phosphorylation in bovine mammary cells. **Physiol. Genomics**, v. 46, p. 268-275, 2014. DOI: 10.1152/physiolgenomics.00119.2013

NARDONE, A.; RONCHI, B.; LACETERA, N.; BERNABUCCI, U. Climatic effects on productive traits in livestock. **Vet. Res. Commun.**, v. 30, p. 75-81, 2006. DOI:10.1007/s11259-006-0016-x

NASEM – NATIONAL ACADEMIES OF SCIENCES, ENGINEERING, AND MEDICINE. **Nutrient requirements of dairy cattle.** 8th rev. ed. Washington, DC: National Academies Press, 2021.

NIU, M.; YING, Y.; BARTELL, P. A.; HARVATINE, K. J. The effects of feeding time on milk production, total-tract digestibility, and daily rhythms of feeding behavior and plasma metabolites and hormones in dairy cows. J. Dairy Sci., v. 97, n. 12, p. 7764-7776, 2014. DOI: 10.3168/jds.2014-8261.

NORDLUND, K. V.; STRASSBURG, P.; BENNETT, T. B.; OETZEL, G. R.; COOK, N. B. Thermodynamics of standing and lying behavior in lactating dairy cows in freestall and parlor holding pens during conditions of heat stress. **J. Dairy Sci.**, v. 102, p. 6495-6507, 2019. DOI: 10.3168/jds.2018-15891

NOUSIAINEN, J.; SHINGFIELD, K. J.; HUHTANEN, P. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. **J. Dairy Sci.**, v. 87, n. 2, p. 386-398, 2004. DOI: 10.3168/jds.S0022-0302(04)73178-1.

OBA, M.; ALLEN, M. S. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci., v. 82, n. 3, p. 589-596, 1999. DOI: 10.3168/jds.S0022-0302(99)75271-9.

OETZEL, G. R. Subacute ruminal acidosis in dairy herds: physiology, pathophysiology, milk fat responses, and nutritional management. In: AMERICAN ASSOCIATION OF BOVINE PRACTITIONERS. 40th Annual Conference. 2007. p. 89-119.

OSORIO, J. S.; JI, P.; DRACKLEY, J. K.; LUCHINI, D., LOOR, J. J. Supplemental smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. **J Dairy Sci.**, v. 96, p. 6248-6263, 2013. DOI: 10.3168/jds.2012-5790

PAGE, T. J.; SIKDER, S.; YANG, L.; PLUTA, L.; WOLFINGER, R. D.; KODADEK, T.; THOMAS, R. S. Genome-wide analysis of human HSF1 signaling reveals a transcriptional program linked to cellular adaptation and survival. **Mol. Biosyst.**, v. 2, p. 627-639, 2006. DOI: 10.1039/b606129j

PAIXÃO, M. G.; ABREU, L. R.; RICHERT, R.; RUEGG, P. L. Milk composition and health status from mammary gland quarters adjacent to glands affected with naturally occurring clinical mastitis. **J. Dairy Sci**., v. 100, n. 9, p. 7522-7533, 2017. DOI: 10.3168/jds.2017-12547.

PALMQUIST, D. L.; BEAULIEU, A. D.; BARBANO, D. M. Feed and animal factors influencing milk fat composition. **J. Dairy Sci.**, v. 76, n. 6, p. 1753-1771, 1993. DOI: 10.3168/jds.S0022-0302(93)77508-6.

PATE, R. T.; LUCHINI, D.; MURPHY, M. R.; CARDOSO, F. C. Effects of rumen-protected methionine on lactation performance and physiological variables during a heat stress challenge in lactating Holstein cows. **J Dairy Sci.**, v. 103(3), p. 2800-2813, 2020. DOI: 10.3168/jds.2019-17305

PATTON, R. A. Effect of rumen-protected methionine on feed intake, milk production, true milk protein concentration, and true milk protein yield, and the factors that influence these effects: A meta-analysis. **J Dairy Sci.**, v. 93, p. 2105-2118, 2010. DOI: 10.3168/jds.2009-2693

PENG, D.; CHEN, S.; LI, G.; CHEN, J.; WANG, J.; GU, X. Infrared thermography measured body surface temperature and its relationship with rectal temperature in dairy cows under different temperature-humidity indexes. **Int. J. Biometeorol.**, v. 63, p. 327-336, 2019. DOI: 10.1007/s00484-018-01666-x

PICCIONE, G.; MESSINA, V.; SCIANÒ, S.; ASSENZA, A.; OREFICE, T.; VAZZANA, I.; ZUMBO, A. Annual changes of some metabolical parameters in dairy cows in the Mediterranean area. **Vet. Arh.**, v. 82, n. 3, p. 229-238, 2012.

PINTO, S.; HOFFMANN, G.; AMMON, C.; AMON, T. Critical THI thresholds based on the physiological parameters of lactating dairy cows. **J. Therm. Biol.**, v. 88, p. 102523, 2020. DOI: 10.1016/j.jtherbio.2020.102523

PIRKKALA, L.; NYKÄNEN, P.; SISTONEN, L. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. **FASEB J.**, v. 15, p. 1118-1131, 2001. DOI: 10.1096/fj00-0294rev

PRAGNA, P.; ARCHANA, P. R.; ALEENA, J.; SEJIAN, V.; KRISHNAN, G.; BAGATH, M.; MANIMARAN, A.; BEENA, V.; KURIEN, E. K.; VARMA, G.; BHATTA, R. Heat stress and dairy cow: Impact on both milk yield and composition. **Int. J. Dairy Sci.**, v. 12, p. 1-11, 2017. DOI: 10.3923/ijds.2017.1.11

PREDIERI, G.; ELVIRI, L.; TEGONI, M.; ZAGNONI, I.; CINTI, E.; BIAGI, G.; FERRUZZA,

S.; LEONARDI G. Metal chelates of 2-hydroxy-4-methylthiobutanoic acid in animal feeding. Part 2: further characterizations, in vitro and in vivo investigations. **J Inorg Biochem.** v. 99, p. 627-636, 2005. DOI: 10.1016/j.jinorgbio.2004.11.011

PSZCZOLKOWSKI, V. L.; HU, H.; ZHANG, J.; CONNELLY, M. K.; MUNSTERMAN, A. S.; ARRIOLA APELO, S. I. Effects of methionine, leucine, and insulin on circulating concentrations and mammary extraction of energy substrates and amino acids in lactating dairy cows. **Domest Anim Endocrinol**. v.8, art. 106730, 2022. DOI: 10.1016/j.domaniend.2022.106730

QU, M.; WEI, S.; CHEN, Z.; WANG, G.; ZHENG, Y.; YAN, P. Differences of hormones involved in adipose metabolism and lactation between high and low producing Holstein cows during heat stress. **Anim. Nutr.**, v. 1, p. 339–343, 2015. DOI: 10.1016/j.aninu.2015.11.003

RHOADS, R. P.; KIM, J. W.; VAN AMBURGH, M. E.; EHRHARDT, R. A.; FRANK, S. J. BOISCLAIR, Y. R. Effect of nutrition on the growth hormone responsiveness of liver and adipose tissue in dairy cows. **J. Endocrinol.**, v. 195, p. 49-58, 2007. DOI: 10.1677/JOE-07-0068

RHOADS, M.L.; RHOADS, R.P.; VANBAALE, M.J.; COLLIER, R.J.; SANDERS, S.R.; WEBER, W.J.; CROOKER, B.A.; BAUMGARD, L.H. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. **J. Dairy Sci.**, v. 92, p. 1986–1997, 2009. DOI: 10.3168/jds.2008-1641

RÍUS, A. G. Invited Review: Adaptations of protein and amino acid metabolism to heat stress in dairy cows and other livestock species. **Appl. Anim. Sci.**, v. 35, p. 39-48, 2019. DOI: 10.15232/aas.2018-01805

ROTH, Z. Reproductive physiology and endocrinology responses of cows exposed to environmental heat stress - Experiences from the past and lessons for the present. **Theriogenology**, v. 155, p. 150-156, 2020. DOI: 10.1016/j.theriogenology.2020.05.040

RUNGRUANG, S.; COLLIER, J. L.; RHOADS, R. P.; BAUMGARD, L. H.; DE VETH, M. J.; COLLIER, R. J. A dose-response evaluation of rumen-protected niacin in thermoneutral or heat-stressed lactating Holstein cows. **J. Dairy Sci**., v. 97, n. 8, p. 5023-5034, 2014. DOI: 10.3168/jds.2013-6970.

SAKATANI, M.; BALBOULA, A. Z.; YAMANAKA, K.; TAKAHASHI, M. Effect of summer heat environment on body temperature, estrous cycles and blood antioxidant levels in Japanese Black cow. **Anim. Sci. J.**, v. 83, p. 394-402, 2012. DOI: 10.1111/j.1740-0929.2011.00967.x

SALAMA, A. A. K.; CONTRERAS-JODAR, A.; LOVE, S.; MEHABA, N.; SUCH, X.; CAJA, G. Milk yield, milk composition, and milk metabolomics of dairy goats intramammary-challenged with lipopolysaccharide under heat stress conditions. **Sci. Rep.**, , v. 10, p. 5055, 2020. DOI: 10.1038/s41598-020-61900-8

SALFER, I. J.; DECHOW, C. D.; HARVATINE, K. J. Annual rhythms of milk and milk fat and protein production in dairy cattle in the United States. **J. Dairy Sc**i., v. 102, n. 1, p. 742-753, 2019. DOI: 10.3168/jds.2018-15040.

SALFER, I. J.; BARTELL, P. A.; DECHOW, C. D.; HARVATINE, K. J. Annual rhythms of milk synthesis in dairy herds in 4 regions of the United States and their relationships to environmental indicators. **J. Dairy Sci**., v. 103, n. 4, p. 3696-3707, 2020. DOI: 10.3168/jds.2019-17362.

SAMMAD, A.; WANG, Y. J.; UMER, S.; LIRONG, H.; KHAN, I.; KHAN, A.; AHMAD, B.; WANG, Y. Nutritional Physiology and Biochemistry of Dairy Cattle under the Influence of Heat Stress: Consequences and Opportunities. **Animals**., v. 10, p. 793, 2020. DOI: 10.3390/ani10050793

SANTOS, M. V.; FONSECA, L. F. L. **Controle da mastite e qualidade do leite: desafios e soluções.** Pirassununga, SP: Edição dos Autores, 2019.

SCHNEIDER, P. L.; BEEDE, D. K.; WILCOX, C. J. Nycterohemeral Patterns of Acid-Base Status, Mineral Concentrations and Digestive Function of Lactating Cows in Natural or Chamber Heat Stress Environments. **J. Anim. Sci.**, v. 66, p. 112-125, 1988. DOI: 10.2527/jas1988.661112x

SCHWAB, C. G.; BRODERICK, G. A. A 100-Year Review: Protein and amino acid nutrition in dairy cows. **J. Dairy Sci.**, v. 100, p. 10094-10112, 2017. DOI: 10.3168/jds.2017-13320

SEJIAN, V.; INDU, S.; NAQVI, S. M. K. Impact of short term exposure to different environmental temperature on the blood biochemical and endocrine responses of Malpura ewes under semi-arid tropical environment. **Indian J. Anim. Sci.**, v. 83, p. 1155–1159, 2013.

SELYE H. Studies on adaptation. **Endocrinology**., v.21, p.169-188, 1937. DOI: 10.1210/endo-21-2-169

SHWARTZ, G.; RHOADS, M .L.; VANBAALE, M. J.; RHOADS, R. P.; BAUMGARD, L. H. Effects of a supplemental yeast culture on heat-stressed lactating Holstein cows. **J. Dairy Sci.**, v. 92, p. 935-942, 2009. DOI: 10.3168/jds.2008-1496

SICILIANO-JONES, J. L.; SOCHA, M.T.; TOMLINSON, D. J.; DEFRAIN, J. M.; Effect of trace mineral source on lactation performance, claw integrity, and fertility of dairy cattle. **J Dairy Sci.**, v. 91, p. 1985-1995, 2008. DOI: 10.3168/jds.2007-0779. PMID: 18420629

SILANIKOVE, N.; MALTZ, E.; SHINDER, D.; BOGIN, E.; BASTHOLM, T.; CHRISTENSEN, N. J.; NORGGARRD, P. Metabolic and productive responses of dairy cows to increased ion supplementation at early lactation in warm Weather. **J. Dairy Res.**, v. 65, p. 529-543, 1998. DOI: 10.1017/s0022029998003185

SILANIKOVE, N.; SHAPIRO, F.; SHINDER, D. Acute heat stress brings down milk secretion in dairy cows by up-regulating the activity of the milk-borne negative feedback regulatory system. **BMC Physiol.**, v. 9, p. 13, 2009. DOI: 10.1186/1472-6793-9-13

SMITH, D. L.; SMITH, T.; RUDE, B. J.; WARD, S. H. Short communication: Comparison of the effects of heat stress on milk and component yields and somatic cell score in Holstein and Jersey cows. **J Dairy Sci.**, v. 96, p. 3028-3033, 2013. DOI: 10.3168/jds.2012-5737

SOBHANIRAD, S.; CARLSON, D.; BAHARI KASHANI, R. Effect of zinc methionine or zinc sulfate supplementation on milk production and composition of milk in lactating dairy cows. **Biol Trace Elem Res.** v. 136, p. 48-54, 2010. DOI: 10.1007/s12011-009-8526-3

SONNA, L. A.; FUJITA, J.; GAFFIN, S. L.; LILLY, C. M. Effects of heat and cold stress on mammalian gene expression. **J. Appl. Physiol.**, v. 92, p. 1725-1742, 2002. DOI: 10.1152/japplphysiol.01143.2001

SORDILLO, L. M., RAPHAEL, W. Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. **Vet Clin N Am Food Anim Pract.,** v. 29, p. 267-278, 2013. DOI: 10.1016/j.cvfa.2013.03.002

SPIERS, D. E.; SPAIN, J. N.; SAMPSON, J. D.; RHOADS, R. P. Use of physiological parameters to predict milk yield and feed intake in heat-stressed dairy cows. **J. Therm. Biol**., v. 29, n. 7-8, p. 759-764, 2004. DOI: 10.1016/j.jtherbio.2004.08.051.

ST-PIERRE, N. R.; COBANOV, B.; SCHNITKEY, G. Economic losses from heat stress by US livestock industries. J. Dairy Sci., v. 86, Suppl., p. E52-E77, 2003. DOI: 10.3168/jds.S0022-0302(03)74040-5.

STARKIE, R.L.; HARGREAVES, M.; ROLLAND, J.; FEBBRAIO, M.A. Heat stress, cytokines, and the immune response to exercise. **Brain. Behav. Immun.**, v. 19, p. 404-412, 2005. DOI: 10.1016/j.bbi.2005.03.005

STEFANSKA, B.; CZŁAPA, W.; PRUSZYNSKA-OSZMAŁEK, E.; SZCZEPANKIEWICZ, D.; FIEVEZ V.; KOMISAREK, J.; STAJEK, K.; NOWAK, W. Subacute ruminal acidosis affects fermentation and endotoxin concentration in the rumen and relative expression of the CD14/TLR4/MD2 genes involved in lipopolysaccharide systemic immune response in dairy cows. **J Dairy Sci.**, v. 101, p. 1297–1310, 2018. DOI: 10.3168/jds.2017-12896

SUDEKUM, K. H.; WOLFFRAM, S.; ADER, P.; ROBERT, J. C. Bioavailability of three ruminally protected methionine sources in cattle. **Anim. Feed Sci. Technol.**, v. 113, p. 17-25, 2004. DOI: 10.1016/j.anifeedsci.2003.11.001

TAO, B. S.; BERNARD, J. K.; MONTEIRO, A. P. A. Cow behavior : A critical factor to consider under heat stress. **UGA Extension Bulletin**, v. 10, p. 1-4, 2015.

THORTON, P.; NELSON G, MAYBERRY D, HERRERO M. Increases in extreme heat stress in domesticated livestock species during the twenty-first century. **Glob Chang Biol.** v. 27, p. 5762-5772, 2021. DOI: 10.1111/gcb.15825

TOLEDO, M. Z.; STANGAFERRO, M. L.; GENNARI, R. S.; BARLETTA, R. V.; PEREZ, M. M.; WIJMA, R.; SITKO, E. M.; GRANADOS, G.; MASELLO, M.; VAN AMBURGH, M. E.; LUCHINI, D.; GIORDANO, J. O.; SHAVER, R. D.; WILTBANK, M. C.. Effects of feeding rumen-protected methionine pre- and postpartum in multiparous Holstein cows: lactation performance and plasma amino acid concentrations. **J Dairy Sci.**, v. 104, p. 7583-7603, 2021. DOI: 10.3168/jds.2020-19021

VERNEQUE, R. S.; REIS FILHO, J. C.; MARTINEZ, M. L.; LOPES, P. S.; TEODORO, R. L.; TORRES, R. A.; MACHADO, M. A.; PEIXOTO, M. G. C. D. Population genetic structure

of Brazilian Gir Dairy Cattle. In: **WORLD CONGRESS ON GENETICS APPLIED TO LIVESTOCK PRODUCTION**, 8., 2006, Belo Horizonte. Proceedings… Belo Horizonte: Instituto Prociência, 2006. p. 01-91.

VIDAL, A. M.; NETTO, A. S. Boas práticas de manejo e impacto na qualidade do leite. In: **CONGRESSO BRASILEIRO DE QUALIDADE DO LEITE**, 10., 2024, Florianópolis. Anais… Florianópolis: CBQL, 2024. p. 203-210.

VITALI, A.; SEGNALINI, M.; BERTOCCHI, L.; BERNABUCCI, U.; NARDONE, A.; LACETERA, N. Seasonal pattern of mortality and relationships between mortality and temperature-humidity index in dairy cows. **J Dairy Sci.**, v. 92, p. 3781-3790, 2009. DOI: 10.3168/jds.2009-2127

VON BORELL, E.H. The biology of stress and its application to livestock housing and transportation assessment. **J. Anim. Sci.**, v. 79, p. 260-267, 2001, DOI: 10.2527/jas2001.79E-SupplE260x

WANG, C.; BEEDE, D. K. Effects of supplemental protein on acid-base status and calcium metabolism of nonlactating jersey cows. **J Dairy Sci.**, v. 73, p. 3178-3186, 1990. DOI: 10.3168/jds.S0022-0302(90)79008-X

WANG, J. P.; BU, D. P.; WANG, J. Q.; HUO, X. K.; GUO, T. J.; WEI, H. Y.; ZHOU, L. Y.; RASTANI, R. R.; BAUMGARD, L. H.; LI, F. D. Effect of saturated fatty acid supplementation on production and metabolism indices in heat-stressed mid-lactation dairy cows. **J Dairy Sci.**, v. 93, p. 4121-4127, 2010. DOI: 10.3168/jds.2009-2635

WEBSTER, J. R.; MOENTER, S. M.; WOODFILL, C. J.; KARSH, F. J. Role of the thyroid gland in seasonal reproduction. II. Thyroxine allows a season-specific suppression of gonadotropin secretion in sheep. **Endocrinology**, v. 129, n. 1, p. 176-183, 1991. DOI: 10.1210/endo-129-1-176.

WENG, X.; MONTEIRO, A. P. A.; GUO, J.; LI, C.; ORELLANA, R. M.; MARINS, T. N.; BERNARD, J. K.; TOMLINSON, D. J.; DEFRAIN, J. M.; WOHLGEMUTH, S. E.; TAO S. Effects of heat stress and dietary zinc source on performance and mammary epithelial integrity of lactating dairy cows. **J Dairy Sci.**, v. 101, p.2617-2630, 2018. DOI: 10.3168/jds.2017-13484

WEST, J. W. Effects of heat-stress on production in dairy cattle. **J. Dairy Sci.**, v. 86, p. 2131-2144, 2003. DOI: 10.3168/jds.S0022-0302(03)73803-X

WHEELOCK, J.; RHOADS, R.; VANBAALE, M.; SANDERS, S.; BAUMGARD, L. Effects of heat stress on energetic metabolism in lactating Holstein cows. **J. Dairy Sci.**, v. 93, p. 644–655, 2010. Doi: 10.3168/jds.2009-2295

WILCOX, C. J.; WEBB, D. W.; DELORENZO, M. A. **Genetic improvement of dairy cattle.** Gainesville: University of Florida, Institute of Food and Agricultural Sciences, Cooperative Extension Service, EDIS, 1992.

WILDRIDGE, A. M.; THOMSON, P. C.; GARCIA, S. C.; JOHN, A. J.; JONGMAN, E. C.; CLARK, C. E. F.; KERRISK, K. L. Short communication: The effect of temperature-humidity index on milk yield and milking frequency of dairy cows in pasture-based automatic milking

systems. **J. Dairy Sci.**, v. 101, p. 4479–4482, 2018. DOI: 10.3168/jds.2017-13867

WOOLPERT, M. E.; DANN, H. M.; COTANCH, K. W.; MELILLI, C.; CHASE, L. E.; GRANT, R. J.; BARBANO, D. M. Management practices, physically effective fiber, and ether extract are related to bulk tank milk de novo fatty acid concentration on Holstein dairy farms. **J. Dairy Sci**., v. 100, n. 6, p. 5097-5106, 2017. DOI: 10.3168/jds.2016-12046.

YOUSEF, M. K. **Stress physiology in livestock**. 1 ed. CRC Press Inc., 1985 p. Boca Raton, FL. 1985.

ZANELA, M. B. et al. Ocorrência e características do leite instável não ácido (LINA) no sul do RS. In: **CONGRESSO BRASILEIRO DE QUALIDADE DO LEITE**, 10., 2024, Florianópolis. Anais… Florianópolis: CBQL, 2024. p. 95-100.

ZANTON, G. I.; BOWMAN, G. R.; VAZQUEZ-ANON, M.; RODE, L. M. Meta-analysis of lactation performance in dairy cows receiving supplemental dietary methionine sources or postruminal infusion of methionine. **J Dairy Sci.**, v. 97, p.7085-7101, 2014. DOI: 10.3168/jds.2014-8220

ZEBELI, Q.; ASCHENBACH, J. R.; TFAJ, M.; BOGUHN, J.; AMETAJ, B. N.; DROCHNER, W. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. **J. Dairy Sci.**, v. 95, n. 3, p. 1041-1056, 2012. DOI: 10.3168/jds.2011-4421.

ZHOU, Z.; VAILATI-RIBONI, M.; TREVISI, E.; DRACKLEY, J. K.; LUCHINI, D. N.;, LOOR, J. J. Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the peripartal period. **J Dairy Sci.**, v. 99, p. 8716-8732, 2016. DOI: 10.3168/jds.2015-10525

ZHU, Y.; LIU, G.; DU, X.; SHI, Z.; JIN, M.; SHA, X.; LI, X.; WANG, Z.; LI, X. Expression patterns of hepatic genes involved in lipid metabolism in cows with subclinical or clinical ketosis. **J. Dairy Sci.**, v. 102, p. 1725-1735, 2018. DOI: 10.3168/jds.2018-14965

ZIMBELMAN, R. B.; RHOADS, R. P.; RHOADS, M. L.; DUFF, G.; BAUMGARD, L. H.; COLLIER, R. J. A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. In: **Proceedings of the Southwest Nutrition Conferenc**e. p. 158-169. 2009.